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

Emmanuel Bäckryd[a,*], Anne-Li Lind[b], Måns Thulin[c], Anders Larsson[d], Björn Gerdle[a], Torsten Gordh[b]

Abstract

Animal models suggest that chemokines are important mediators in the pathophysiology of neuropathic pain. Indeed, these substances have been called "gliotransmitters," a term that illustrates the close interplay between glial cells and neurons in the context of neuroinflammation and pain. However, evidence in humans is scarce. The aim of the study was to determine a comprehensive cerebrospinal fluid (CSF) inflammatory profile of patients with neuropathic pain. Our hypothesis was that we would thereby find indications of a postulated on-going process of central neuroinflammation. Samples of CSF were collected from 2 cohorts of patients with neuropathic pain (n = 11 and n = 16, respectively) and healthy control subjects (n = 11). The samples were analyzed with a multiplex proximity extension assay in which 92 inflammation-related proteins were measured simultaneously (Proseek Multiplex Inflammation I; Olink Bioscience, Uppsala, Sweden). Univariate testing with control of false discovery rate, as well as orthogonal partial least squares discriminant analysis, were used for statistical analyses. Levels of chemokines CXCL6, CXCL10, CCL8, CCL11, CCL23 in CSF, as well as protein LAPTGF-beta-1, were significantly higher in both neuropathic pain cohorts compared with healthy controls, pointing to neuroinflammation in patients. These 6 proteins were also major results in a recent similar study in patients with fibromyalgia. The findings need to be confirmed in larger cohorts, and the question of causality remains to be settled. Because it has been suggested that prevalent comorbidities to chronic pain (eg, depression, anxiety, poor sleep, and tiredness) also are associated with neuroinflammation, it will be important to determine whether neuroinflammation is a common mediator.

Keywords: Biomarker, Cerebrospinal fluid, Chemokines, Cytokines, Human, Inflammation, Neuroinflammation, Neuropathic pain, Protein profile, Proximity extension assay

1. Introduction

Neuropathic pain (NeuP) is defined as pain caused by a lesion or disease of the somatosensory nervous system.[33] The prevalence of chronic pain with neuropathic characteristics in the general population has been estimated to be up to 7%.[8] Available analgesics often have limited effects or lead to troublesome side effects.[5,27] Current evidence indicates that at least 6 patients have to be treated with a first-line drug (eg, serotonin–norepinephrine reuptake inhibitors or gabapentinoids) in order for 1 patient to obtain clinically significant pain relief.[27]

When trying to better understand what causes and maintains NeuP, it is important to move beyond mere etiology and study the pathophysiological mechanisms involved, for instance by in-depths somatosensory phenotyping.[70] Another way forward is to study the biochemical profile of NeuP patients using 'omics methodology.[2,10,64,79] The cerebrospinal fluid (CSF) seems to be a sensible biofluid to investigate in pain conditions, as it can reasonably be hypothesized to mirror central nervous system pathology. For instance, CSF levels of classical neuropeptides, like substance P and beta-endorphin (and other endogenous opioids), have historically been studied in many different pain states.[1,4,11,65,73,74]

Much of our knowledge concerning the pathophysiological mechanisms of NeuP has been gained from animal experiments. It has become increasingly clear that immunocompetent glial cells, such as microglia and astrocytes, are key contributors to the pathophysiology of chronic NeuP.[6,14,24,31,32,51,60,76,78] Hence, central neuroimmune and neuroinflammatory mechanisms are nowadays considered to be very important in the pathophysiology of NeuP. However, it is important to stress that this has mainly been shown in preclinical models of chronic pain and that evidence in humans is less clear.[14,32,60] Indeed, glial cells (at least astrocytes) from mice and monkeys are quite different from their human counterparts.[71] Translating evidence from animals to

humans is far from trivial,[50] but a series of recent studies using a comprehensive panel of 92 inflammation-related proteins indicate the presence of low-grade systemic inflammation and neuroinflammation in chronic widespread pain conditions[13,30] and of systemic inflammation in chronic lumbar radicular pain.[55]

Cytokines and chemokines are thought to be important mediators in the pathophysiology of NeuP, at least in preclinical models.[18,42,60] Indeed, chemokines and other pronociceptive mediators in the spinal cord have been called "gliotransmitters,"[60] a term that illustrates the close interplay between glial cells and neurons in the context of neuroinflammation and chronic pain.

The aim of the present study was to use a multiplex panel allowing the measuring of 92 inflammation-related proteins in a single run[55] and apply it to the CSF of patients with peripheral NeuP and healthy control subjects. Our hypothesis was that we would be able to determine a CSF inflammatory profile of NeuP patients and that we would be able to mirror a postulated on-going process of central neuroinflammation.

2. Methods

First, we compared NeuP patients (called cohort 1a) and healthy control subjects (cohort 1b) recruited at the same center (Linköping, Sweden). Then, to test the reproducibility of our results, an additional cohort of patients (called cohort 2) with a similar pain condition but belonging to another center (Uppsala, Sweden) were compared with cohort 1b.

2.1. Procedures

For every subject in this study, intrathecal access was obtained by lumbar puncture and a 10-mL sample of CSF was taken. Details of the CSF sampling procedure have been published earlier and are not repeated here.[10,46]

2.2. Subjects

Following the criteria of Treede et al.,[72] all patients in cohort 1a had a least probable NeuP and patients in cohort 2 had definite NeuP. All cohort 1a patients included in this study were also participating in a clinical trial of intrathecal bolus injections of the analgesic ziconotide, CSF being sampled *before* the injection of ziconotide.[12] Inclusion criteria were as follows: (1) patient, at least 18 years of age, experiencing chronic (≥6 months) NeuP due to trauma or surgery, who had failed on conventional pharmacological treatment; (2) average visual analogue scale pain intensity in the previous week of ≥40 mm; (3) patient capable of judgment, that is, able to understand information regarding the drug, the mode of administration, and evaluation of efficacy and side effects; (4) signed informed consent. Exclusion criteria and other registered characteristics have been extensively described elsewhere.[10] All patients were or had been candidates for spinal cord stimulation (SCS).

Age-matched and sex-matched healthy control subjects (cohort 1b) were recruited by local advertisements at the Faculty of Health Sciences, Linköping University, Sweden.[10]

Cohort 2 consisted of patients being treated with SCS. The SCS treatment was turned off during 2 days, whereupon a lumbar puncture was performed. Details about cohort 2 have been published elsewhere.[46]

2.3. Proximity extension assay

We used a multiplex proximity extension assay (PEA) in which 92 proteins (see supplemental digital content 1, available

online at http://links.lww.com/PAIN/A482) are simultaneously analyzed.[3,47,55] The multiplex PEA was conducted using Proseek Multiplex Inflammation I (Olink Bioscience, Uppsala, Sweden) according to the manufacturer's instructions. Briefly, 1-μL sample was mixed with 3-μL incubation mixture containing 94 probe pairs (each pair consisting of 2 target-specific antibodies equipped with unique barcoded DNA oligonucleotides). The mixture was incubated at 8°C overnight. Then, 96-μL extension mixture containing PEA enzyme and polymerase chain reaction reagents was added, incubated for 5 minutes at room temperature before the plate was transferred to a thermal cycler for an extension reaction followed by 17 cycles of DNA amplification. A 96.96 Dynamic Array IFC (Fluidigm, South San Francisco, CA) was prepared and primed according to the manufacturer's instructions. In a new plate, 2.8 μL of sample mixture was mixed with 7.2 μL of detection mixture from which 5 μL was loaded into the right side of the primed 96.96 Dynamic Array IFC. Five microliters of the primer pairs, unique for each assay, was loaded into the left side of the 96.96 Dynamic Array IFC, and the protein expression program was run in Fluidigm Biomark reader (Fluidigm Corporation) according to the instructions of Proseek Multiplex. Data are expressed as normalized protein expression (NPX). Values of NPX are acquired by normalizing cq values against extension control, as well as interplate control and a correction factor. They are on log2 scale. A high NPX value corresponds to a high protein concentration and can be linearized using the formula 2^{NPX}. Also, NPX can be used for statistical multivariate analysis and express relative quantification between samples but is not an absolute quantification. Data showing the correlation between the present PEA method and an electrochemiluminescence immunoassay (Meso Scale Discovery MULTI-ARRAY technology) for plasma CXCL1 and CXCL10 is shown in supplemental digital content 1 (available online at http://links.lww.com/PAIN/A482), where a link to the extensive background information on the method available online is also provided.

2.4. Statistics

When comparing the demographics of patients and healthy control subjects, data are shown as median (range), and the Mann–Whitney U test or Fisher exact test was used as appropriate for inferential statistics (version 23, IBM SPSS statistics, IBM Corporation, Armonk, NY).

Proteins with more than 20% of values below the limit of detection were excluded from further analysis.[55] For each protein, we tested whether there was a difference in expression levels between the 2 groups using a 2-sided Mann–Whitney U test. Performing such a large number of tests increases the risk of false discoveries. Therefore, we adjusted the P values for multiplicity using the false discovery rate (FDR) approach.[7]

We also used multivariate data analysis by projection with the SIMCA software version 13 (Umetrics AB, Umeå, Sweden).[10,25,79] The statistical workflow has been extensively described elsewhere,[10,59] and it is consistent with the recommendations issued by Wheelock and Wheelock.[79] Multivariate data analysis by projection analyzes all the variables together at the same time, taking the correlation structure of the data set into consideration, thereby favoring structure and information over "noise" and false-positive findings.[25] Briefly, data were first overviewed by principal components analysis (PCA) (which conceptually can be viewed as a multivariate correlation analysis). However, PCA was used here for the identification of outliers and deviant subgroups in the data. Then, orthogonal partial least squares discriminant

analysis (OPLS-DA) (ie, a regression technique) was used to identify the proteins (ie, X variables) most responsible for class discrimination (dichotomous Y variable). The statistical significance of the regression is expressed using the P value of the cross-validated analysis of variance (CV-ANOVA). The strength of class separation can be visualized in a plot showing how each individual subject relates to the 2 first latent variables of the model (score plot). The relative importance of each protein (X variable) for class discrimination is given by the variable influence on projection (VIP), VIP >1 indicating that the variable has an above-average influence on class discrimination (Y variable).[25] In this study, a VIP cutoff of 1.3 was chosen for reporting interesting class-discriminating proteins.

2.5. Ethics

The protocol of healthy control subjects was approved by the Regional Ethics Committee in Linköping, Sweden (Dnr M136-06 and Dnr 2012/94-32). The clinical trial, from which patient data from Linköping were derived, was conjointly approved by the Swedish Medical Products Agency (EudraCT 2010-018,920-21) and by the Regional Ethics Committee in Linköping (Dnr 2011/48-31). The study was also approved by the Regional Ethics Committee in Uppsala (01-367). The study was conducted according to the Declaration of Helsinki.

3. Results

3.1. Patients with neuropathic pain (cohort 1a) versus healthy control subjects (cohort 1b)

Patients with NeuP (cohort 1a, n = 11) and healthy control subjects (cohort 1b, n = 11) did not differ significantly concerning age (57 [39-65] years vs 54 [44-57] years, respectively; $P = 0.088$) and sex (55% vs 64% women, respectively; $P = 1.0$). For detailed individual characteristics of patients of cohort 1a, see **Table 1**.

Multiple univariate tests with control of the FDR: 42 of 92 inflammation-related proteins had more than 20% of values below limit of detection and were therefore excluded from analysis. Hence, the following results pertain to the levels of 50 inflammation-related proteins. At an FDR of 10%, the following inflammation-related proteins were significantly associated with NeuP when comparing cohorts 1a and 1b: CXCL1, CXCL5, CXCL6, CXCL10, CCL3, CCL8, CCL11, CCL19, CCL23, LAPTGF-beta-1, and LIF-R (**Table 2** and **Fig. 1**). Nine of these 11 proteins were chemokines.

Cohorts 1a and 1b were overviewed with PCA (2 principal components, $R^2 = 0.62$, $Q^2 = 0.46$); no outlier was found. Then, an OPLS-DA model was computed (1 predictive intraclass latent variable and 1 interclass latent variable, $R^2 = 0.67$ and $Q^2 = 0.43$), showing clear separation between patients and healthy control subjects ($P = 0.038$ by CV-ANOVA; **Fig. 2**). Eleven proteins had VIP of >1.3 (ie, were very important for group discrimination), and these were the same as the ones listed above using the FDR; the VIP values of OPLS-DA are shown in **Table 2**.

3.2. Patients with neuropathic pain (cohort 2) versus healthy control subjects (cohort 1b)

The cohort 2 patients (n = 16) did not significantly differ from the cohort 1b healthy controls (n = 11) concerning age (56 [46-68] years vs 54 [44-57] years, respectively; $P = 0.178$) and sex (69% vs 64% women, respectively; $P = 1.0$). Detailed individual characteristics for cohort 2 are shown in **Table 3**. Data on 50 inflammation-related proteins were available.

Cohorts 2 and 1b were overviewed with PCA (2 principal components, $R^2 = 0.69$, $Q^2 = 0.57$); no outlier was found. An OPLS-DA model was computed for cohort 2 versus cohort 1b (1 predictive intraclass latent variable and 2 interclass latent variables, $R^2 = 0.90$ and $Q^2 = 0.66$; $P < 0.001$ by CV-ANOVA). Eleven proteins had VIP of >1.3 and were upregulated in patients: LAPTGF-beta-1, CCL11, 4E-BP1, CXCL10, CCL23, CX3CL1, CXCL6, CD5, CCL8, CCL25, and CXCL11 (in falling order of VIP, range, 1.75-1.31).

3.3. Overlap between the 2 neuropathic pain cohorts

We found a 55% overlap when comparing the top 11 proteins of the 2 OPLS-DA models: LAPTGF-beta-1, CCL11, CXCL10,

Table 1

Characteristics of patients with neuropathic pain for cohort 1a (n = 11).

Main cause of pain *ICD-10*	Pain duration (mo)	VASPI	Concomitant analgesics	Concomitant OME (mg/d)	Comorbidities
S14.2	18	84		0	Hypertension
S34.2	36	87	P	0	Anemia; dyspepsia; hypertension
S34.2	120	40		0	None
S34.3	79	78	P, NSAID, AD, Gab, Op	480	None
S34.2	180	71	AD, Gab, Op	32	Autonomic neuropathy; diabetes; dyspepsia; mild angina; panic anxiety disorder
S34.2	48	83	P, Gab, Op	50	Localized bladder tumor
S34.2	120	74	P, Op	20	Depression
S34.2	120	75		0	Alcohol dependence; polyneuropathy; psoriasis; tension headache
S54.9	300	64	P, Op	30	None
G62.9	78	68		0	None
S14.2	18	58	AD, Gab, Op	30	None

International Classification of Diseases (ICD-10) key: S14.2, injury of nerve root of cervical spine; S34.2, injury of nerve root of lumbar and sacral spine (ie, failed back surgery syndrome with radiculopathy); S34.3, injury of cauda equina; S54.9, injury of unspecified nerve at forearm level.

AD, amitriptyline or duloxetine; Gab, gabapentinoids; NSAID, nonsteroidal anti-inflammatory drug; OME, oral morphine equivalents; Op, opioids; P, paracetamol; VASPI, visual analogue scale for pain intensity last week (0-100 mm).

List of upregulated inflammation-related proteins in the cerebrospinal fluid of patients with neuropathic pain (cohort 1a), compared with healthy control subjects (cohort 1b), by multiple univariate testing with control of FDR and by OPLS-DA.

Protein	UniProt no.	Increase (%)	*P*	FDR, q-value	OPLS-DA, VIP
CXCL6	P80162	59	<0.001	0.014	1.7
CXCL10	P02778	67	<0.001	0.014	1.7
LIF-R	P42702	63	0.001	0.024	1.7
CCL23	P55773	56	0.002	0.025	1.7
CXCL5	P42830	55	0.003	0.025	1.6
CCL11	P51671	38	0.004	0.032	1.6
LAP-TGF-beta-1	P01137	54	0.004	0.032	1.6
CXCL1	P09341	46	0.010	0.059	1.5
CCL19	Q99731	91	0.010	0.059	1.5
CCL3/MIP-1- alpha	P10147	32	0.011	0.059	1.4
CCL8/MCP-2	P80075	44	0.011	0.059	1.4

The percentages in the "increase" column indicate descriptively how much larger the median expression levels (in linearized normalized protein expression, NPX) were in the neuropathic pain group. For details about NPX, see Statistics section.

FDR, false discovery rate (a q-value below 0.1 corresponds to there being a significant difference at a FDR of 10%); OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable influence on projection; metric used in the OPLS-DA regression, see statistics section and supplemental digital content 2 (statistical considerations, http://links.lww.com/PAIN/A482).

CCL23, CXCL6, and CCL8 were common to both models. LIF-R, CXCL1, CCL19, CXCL5, and CCL3 were specific for cohort 1a, whereas 4E-BP1, CX3CL1, CD5, CCL25, and CXCL11 were specific for cohort 2. Among the proteins specific for either cohort, the following 5 proteins had VIP 1.0 to 1.3 in the other cohort, that is, were somewhat (albeit not very strongly) associated with NeuP in the other cohort as well: CXCL1, CXCL5, CXCL11, and CX3CL1.

4. Discussion

We have determined an extensive CSF inflammatory profile of patients with severe peripheral NeuP who were candidates for (cohort 1a) or had an on-going (cohort 2) treatment with SCS, compared with healthy control subjects (cohort 1b).

4.1. The question of reproducibility

The same panel has recently been used for serum profiling of NeuP patients,[55] but this is the first time that such a "holistic" CSF inflammatory fingerprint has been described for NeuP. We have also recently used the same panel on CSF from patients with fibromyalgia,[13] with a remarkable overlap of results with the present study: all 6 proteins upregulated in both NeuP cohorts (5 of which were chemokines) were also major findings in patients with fibromyalgia. Even though it has to be acknowledged that the present study and the fibromyalgia study shared the same CSF control group, the overlap of results is nonetheless striking. Recent plasma–serum studies using the same multiplex panel[13,30,55] have also shown remarkable overlaps in results (different cohorts of patients and different control groups). Statistical considerations are discussed in supplemental digital content 2 (available online at http://links.lww.com/PAIN/A482).

A large part of the main findings of cohort 1a could be reproduced in cohort 2, with an overlap of 55% concerning the top 11 proteins. The difference between the top 11 proteins could perhaps be due to the fact that the 2 NeuP cohorts differed concerning the presence or absence of long-term SCS treatment. Even though the 2 cohorts consisted of more or less the same category of patients, it is conceivable that long-term modulatory

effects of SCS might have altered the CSF inflammatory profile of cohort 2. Even though the 2 NeuP cohorts were compared with the same control group, which is an obvious limitation, the actual overlap of results between the 2 comparisons is still noteworthy. Also, both patient cohorts were highly refractory to conventional treatment, and our results cannot be generalized to any NeuP.

Given that levels of 15 of 63 cytokines have been shown to be associated with age (albeit in plasma),[44] the fact that the groups did not differ statistically concerning age and sex is important to underline.

4.2. Chemokines and neuroinflammation

A description of the chemokine family, and of LAPTGF-beta-1, can be found in supplemental digital content 3 (available online at http://links.lww.com/PAIN/A482). Strikingly, levels of LAPTGF-beta-1 have also been increased in all the other studies that we have hitherto performed with the present panel (including the present study).[13,30,55]

Chemokine receptors are potential pharmacological targets.[58] Chemokines can induce NeuP-like behavior in mice via bidirectional neuron–glia interactions.[35] The contribution of spinal chemokines, primarily CCL2 (MCP-1) and CX3CL1 (fractalkine) and also CXCL21, CXCL13 and other chemokines, to pain-like behavior in rodent models of NeuP has been extensively reviewed.[28,36] Notably, CX3CL1 (fractalkine) is thought to be involved in a prominent pathway in the development of NeuP.[18,60] For example, in the spinal cord dorsal horn, CX3CL1 produced by neurons has been shown to interact with microglial CX3CR1, triggering an increased production of proinflammatory cytokines such as tumor necrosis factor-alpha, interleukin (IL)-1b, and IL-6, causing central sensitization and increased pain-like behavior.[28,41] Neuropathic animals have high CSF levels of CX3CL1.[20] Another chemokine, CCL2, activates microglia and directly influences neurons; CCL2 can induce rapid central sensitization of dorsal horn neurons via ERK activation and enhances their excitatory synaptic transmission.[28] The production of CXCL1 by spinal cord astrocytes has been shown to contribute to the maintenance of pain-like behavior in NeuP animal models,[17,81] Moreover, CXCL1 has recently been found to be upregulated in the CSF samples of
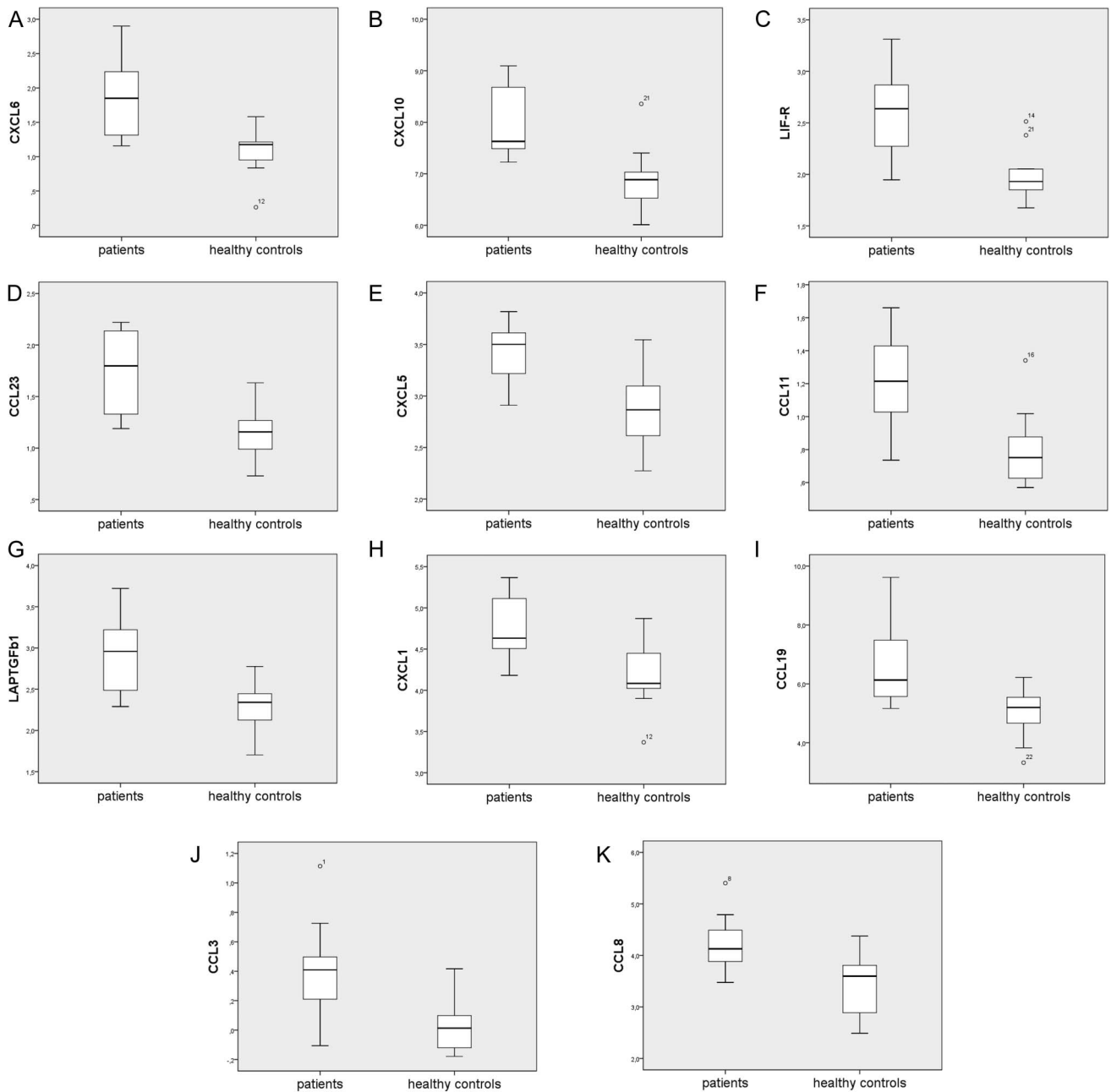
Figure 1. Expression of the 11 most group-discriminating inflammation-related proteins in the cerebrospinal fluid of neuropathic pain patients (cohort 1a) versus healthy control subjects (cohort 1b). The protein levels (y-axis) are expressed as normalized protein expression, as described in the proximity extension assay subsection. Median values are represented by horizontal lines and the interquartile ranges by boxes. The ends of the whiskers depict the lowest and highest datum within 1.5 interquartile range of the lower or upper quartile, respectively. Points represent outliers.

opioid-tolerant cancer patients.[45] Moreover, there are indications that CXCL10 may also be involved in pain-like behavior maintenance in rodent models of NeuP.[16,37,68] Neutralizing the action of chemokines, CCL2 or CX3CL1, attenuates nerve injury–induced pain-like behavior in rodents.[19,29,53,54,69,82] Astrocytic chemokines can then modulate neuronal activity and potentiate synaptic transmission in the spinal cord excitatory pain circuitry.[28] Additional chemokine references are listed in supplemental digital content 3 (available online at http://links.lww.com/PAIN/A482). The findings reported in this study are consistent with a role for chemokines in *human* NeuP.

Given the chemokines mentioned above, it is noteworthy that CX3CL1, CXCL1, and CXCL10 were part of the main findings of the present study:

(1) CX3CL1 was a main finding of cohort 2. It was not part of the main findings of cohort 1a, but a retrospective analysis revealed that it was actually upregulated in that cohort too (median linearized NPX 98% higher in patients; $P < 0.001$; VIP = 1.06) We have previously also shown that levels of CX3CL1 were high in the CSF of patients with fibromyalgia.[13]

(2) CXCL1 was elevated in cohort 1a. It was not part of the main findings of cohort 2, but it was actually upregulated in that cohort too (median linearized NPX 46% higher in patients; $P = 0.009$; VIP = 1.23).

(3) CXCL10 was a main finding in both cohorts.

To the best of our knowledge, among the other major findings of the study, neither CXCL6 nor CCL23 have been implicated in
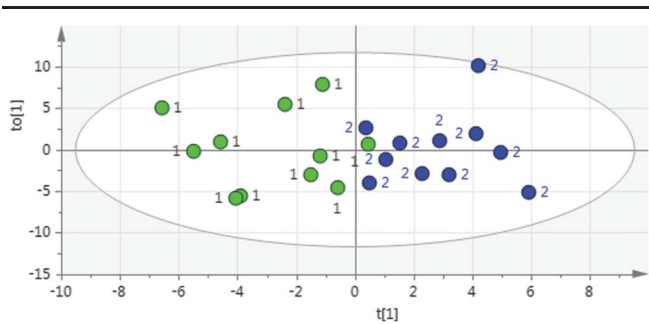
Figure 2. Two-dimensional score plot of orthogonal partial least squares discriminant analysis comparing inflammation-related proteins in the cerebrospinal fluid of patients with neuropathic pain (cohort 1a) with healthy control subjects (cohort 1b). Class separation between neuropathic pain patients (n = 11, green dots marked "1") and healthy controls (n = 11, blue dots marked "2") occurs along the t[1] axis (interclass variation). The to[1] axis represents intraclass variation. The ellipse represents the Hotelling T^2 95% confidence interval used when identifying strong outliers.

NeuP. In contrast, CCL11 has been investigated in at least 3 models of NeuP[43,49,67]; see also supplemental digital content 3 (available online at http://links.lww.com/PAIN/A482).

4.3. Cytokines, neurotrophic factors, and neuroinflammation

"Classical" cytokines (tumor necrosis factor-α, IL-6, IL-1b)[9,31,52,76] and neurotrophic factors[15,48] are discussed in supplemental digital content 3 (available online at http://links.lww.com/PAIN/A482).

4.4. Neuroinflammation and neuropathic pain

Although neuroinflammation is not easily defined,[26,57] it is nonetheless a frequently used concept in modern pain medicine.[24,32] Are we perhaps measuring some aspects of central neuroinflammation in humans? This would be a major

step forward for pain medicine, as evidence of central neuroinflammation has hitherto been mostly gained through animal experiments.[32,76]

Neuroinflammation can be said to have 3 characteristic components with effects on pain behavior in animal models: (1) infiltration of immune cells,[22] (2) activation of glial cells,[21,24,29,40,63,80] and (3) production of inflammatory mediators.[34,36] Neuroinflammation can contribute to central sensitizationand NeuP by chemokine[18] and cytokine pathways.[41]

All in all, we think it is fair to say that we might have "visualized" central neuroinflammation, this being one possible mechanism associated with central sensitization, impaired descending pain inhibition, and the pain hypersensitivity characterizing chronic pain states.[38,39]

4.5. The question of causality

Granted that our results are valid (ie, that they really reflect pain-related pathophysiology and not, eg, a confounding effect of concomitant medicines or of other medical conditions such as the ones listed in **Table 1**), it is important to consider whether the CSF inflammatory fingerprint that we have described *directly* relates to the *pathophysiology* of NeuP (eg, central sensitization due to neuroinflammation) or if it is an inflammatory *risk factor* that was present prior to the development of NeuP (eg, a genetic susceptibility such as HLA haplotype[23,61,66,77]). A third possibility could be that the fingerprint is a *consequence* of NeuP, for example, mirroring pain-related stress, physical inactivity,[62] depression,[75] or bad sleep.[56] Of course, all 3 of these categories may play a role. Disentangling the contribution of these potentially mutually interacting factors will be very difficult. For instance, levels of peripheral IL-6 are known to be influenced by regular exercise, individuals who are inactive having higher baseline levels of this particular cytokine.[62]

It is important to underline that cytokines and chemokines are probably not very specific biomarkers. It seems sensible to hypothesize that, in the future, biomarkers for different chronic

Table 3

Neuropathic pain characteristics for cohort 2 (n = 16).

Study ID	Pain diagnosis	Level of nerve lesion	Painful area	Years with SCS
501	Radiculopathy	L4-5	Leg, back, foot	2
502	Radiculopathy	C5-6-7	Hand, fingers	3
503	Polyneuropathy	Peripheral nerves of the legs	Back, leg, left arm	7
504	Stump and phantom limb pain	Peripheral nerves of the leg	Stump of and left amputated leg	2
506	Radiculopathy	L5-S1	Thigh, left buttock	7
507	Radiculopathy	L4-5	Thigh, back	7
538	Radiculopathy	L4-5	Right leg, lumbar back, right hip, thigh, lower leg	3
510	Radiculopathy	L5-S1	Foot	2
512	Painful scar after pyeloplastic surgery	Spinal nerve (approx. Th10)	Flank	10
513	Radiculopathy, chronic low back pain	L5-S1	Lateral part of the foot	12
514	Radiculopathy	L5-S1	Lower back, leg	5
515	Radiculopathy	Th8	Flank	3
516	Radiculopathy	C4-C6	Arm	0.5
517	Radiculopathy	C4 + lateral antebrachial cutaneous nerve	Arm	7
519	Radiculopathy	L5	Left leg, back	3
550	Radiculopathy	L4-5	Left leg	2

SCS, spinal cord stimulation.

pain conditions may fall into 2 categories, namely, on the one hand those that are common to several or perhaps even all chronic pain conditions and, on the other hand, those that are specific for a given condition. In future CSF studies, it will be important to determine unique and common markers for different pain conditions. Such studies will also have to take comorbid conditions, like depression, anxiety, and poor sleep, into consideration, as these might also be associated with chronic inflammation.[75] That this was not done is a limitation of the study, as is the fact that we did not register factors like the level of physical activity and smoking or alcohol use, and that we did not take putative diurnal variations into consideration when planning the study.

5. Conclusions

Using a panel of inflammation-related proteins, we have found evidence of on-going neuroinflammation in patients with NeuP. The results from 2 cohorts of fairly comparable patients were quite similar (although not perfectly identical), showing that mainly a number of chemokines were upregulated in CSF from patients compared with healthy control subjects. We find it conceivable that we might have mirrored central neuroinflammation in this very debilitating chronic pain condition. However, further studies are needed to confirm these findings, and the question of causality remains difficult to answer. Because it has been suggested that prevalent comorbidities to chronic pain also are associated with neuroinflammation, it will be important to determine unique and common mediators.

Conflict of interest statement

The authors have no conflict of interest to declare.

Acknowledgments

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/A482.

References

[1] Almay BG, Johansson F, Von Knorring L, Le Greves P, Terenius L. Substance P in CSF of patients with chronic pain syndromes. PAIN 1988;33:3–9.

[2] Antunes-Martins A, Perkins JR, Lees J, Hildebrandt T, Orengo C, Bennett DL. Systems biology approaches to finding novel pain mediators. Wiley Interdiscip Rev Syst Biol Med 2013;5:11–35.

[3] Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, Andersson AC, Lindstedt P, Stenvang J, Gullberg M, Fredriksson S. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 2014;9:e95192.

[4] Baraniuk JN, Whalen G, Cunningham J, Clauw DJ. Cerebrospinal fluid levels of opioid peptides in fibromyalgia and chronic low back pain. BMC Musculoskelet Disord 2004;5:48.

[5] Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol 2010;9: 807–19.

[6] Beggs S, Trang T, Salter MW. P2X4R+ microglia drive neuropathic pain. Nat Neurosci 2012;15:1068–73.

[7] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 1995;57:289–300.

[8] Bouhassira D, Lanteri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. PAIN 2008;136:380–7.

[9] Brisby H, Olmarker K, Larsson K, Nutu M, Rydevik B. Proinflammatory cytokines in cerebrospinal fluid and serum in patients with disc herniation and sciatica. Eur Spine J 2002;11:62–6.

[10] Bäckryd E, Ghafouri B, Carlsson AK, Olausson P, Gerdle B. Multivariate proteomic analysis of the cerebrospinal fluid of patients with peripheral neuropathic pain and healthy controls—a hypothesis-generating pilot study. J Pain Res 2015;8:321–33.

[11] Bäckryd E, Ghafouri B, Larsson B, Gerdle B. Do low levels of beta-endorphin in the cerebrospinal fluid indicate defective top-down inhibition in patients with chronic neuropathic pain? A cross-sectional, comparative study. Pain Med 2014;15:111–19.

[12] Bäckryd E, Sorensen J, Gerdle B. Ziconotide trialing by intrathecal bolus injections: an open-label non-randomized clinical trial in postoperative/posttraumatic neuropathic pain patients refractory to conventional treatment. Neuromodulation 2015;18:404–13.

[13] Bäckryd E, Tanum L, Lind AL, Larsson A, Gordh T. Evidence of both systemic inflammation and neuroinflammation in fibromyalgia patients, as assessed by a multiplex protein panel applied to the cerebrospinal fluid and to plasma. J Pain Res 2017;10:515–25.

[14] Calvo M, Dawes JM, Bennett DL. The role of the immune system in the generation of neuropathic pain. Lancet Neurol 2012;11:629–42.

[15] Capelle HH, Weigel R, Schmelz M, Krauss JK. Neurotrophins in the cerebrospinal fluid of patient cohorts with neuropathic pain, nociceptive pain, or normal pressure hydrocephalus. Clin J Pain 2009;25:729–33.

[16] Carr FB, Geranton SM, Hunt SP. Descending controls modulate inflammatory joint pain and regulate CXC chemokine and iNOS expression in the dorsal horn. Mol Pain 2014;10:39.

[17] Chen G, Park CK, Xie RG, Berta T, Nedergaard M, Ji RR. Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice. Brain 2014;137:2193–209.

[18] Clark AK, Malcangio M. Fractalkine/CX3CR1 signaling during neuropathic pain. Front Cell Neurosci 2014;8:121.

[19] Clark AK, Yip PK, Grist J, Gentry C, Staniland AA, Marchand F, Dehvari M, Wotherspoon G, Winter J, Ullah J, Bevan S, Malcangio M. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. Proc Natl Acad Sci U S A 2007;104:10655–60.

[20] Clark AK, Yip PK, Malcangio M. The liberation of fractalkine in the dorsal horn requires microglial cathepsin S. J Neurosci 2009;29:6945–54.

[21] Colburn RW, Rickman AJ, DeLeo JA. The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. Exp Neurol 1999;157:289–304.

[22] Costigan M, Moss A, Latremoliere A, Johnston C, Verma-Gandhu M, Herbert TA, Barrett L, Brenner GJ, Vardeh D, Woolf CJ, Fitzgerald M. T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. J Neurosci 2009;29: 14415–22.

[23] Dominguez CA, Kalliomaki M, Gunnarsson U, Moen A, Sandblom G, Kockum I, Lavant E, Olsson T, Nyberg F, Rygh LJ, Roe C, Gjerstad J, Gordh T, Piehl F. The DQB1 *03:02 HLA haplotype is associated with increased risk of chronic pain after inguinal hernia surgery and lumbar disc herniation. PAIN 2013;154:427–33.

[24] Ellis A, Bennett DL. Neuroinflammation and the generation of neuropathic pain. Br J Anaesth 2013;111:26–37.

[25] Eriksson L, Byrne T, Johansson E, Trygg J, Vikström C. Multi- and megavariate data analysis: basic principles and applications. Malmö: MKS Umetrics AB 2013:1–254.

[26] Estes ML, McAllister AK. Alterations in immune cells and mediators in the brain: it's not always neuroinflammation! Brain Pathol 2014;24: 623–30.

[27] Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, Gilron I, Haanpaa M, Hansson P, Jensen TS, Kamerman PR, Lund K, Moore A, Raja SN, Rice AS, Rowbotham M, Sena E, 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] Gao YJ, Ji RR. Chemokines, neuronal-glial interactions, and central processing of neuropathic pain. Pharmacol Ther 2010;126:56–68.

[29] Gao YJ, Zhang L, Samad OA, Suter MR, Yasuhiko K, Xu ZZ, Park JY, Lind AL, Ma 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] Gerdle B, Ghafouri B, Ghafouri N, Backryd E, Gordh T. Signs of ongoing inflammation in female patients with chronic widespread pain: a multivariate, explorative, cross-sectional study of blood samples. Medicine (Baltimore) 2017;96:e6130.

[31] Gosselin RD, Suter MR, Ji RR, Decosterd I. Glial cells and chronic pain. Neuroscientist 2010;16:519–31.

[32] Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol 2014;14:217–31.

[33] Jensen TS, Baron R, Haanpaa M, Kalso E, Loeser JD, Rice AS, Treede RD. A new definition of neuropathic pain. PAIN 2011;152:2204–5.

[34] Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? PAIN 2013;154(suppl 1):S10–28.

[35] Ji RR, Chamessian A, Zhang YQ. Pain regulation by non-neuronal cells and inflammation. Science 2016;354:572–7.

[36] Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov 2014;13:533–48.

[37] Jiang BC, He LN, Wu XB, Shi H, Zhang WW, Zhang ZJ, Cao DL, Li CH, Gu J, Gao YJ. Promoted interaction of C/EBPalpha with demethylated Cxcr3 gene promoter contributes to neuropathic pain in mice. J Neurosci 2017;37:685–700.

[38] Kadetoff D, Lampa J, Westman M, Andersson M, Kosek E. Evidence of central inflammation in fibromyalgia-increased cerebrospinal fluid interleukin-8 levels. J Neuroimmunol 2012;242:33–8.

[39] Karshikoff B, Jensen KB, Kosek E, Kalpouzos G, Soop A, Ingvar M, Olgart Hoglund C, Lekander M, Axelsson J. Why sickness hurts: a central mechanism for pain induced by peripheral inflammation. Brain Behav Immun 2016;57:38–46.

[40] Katsura H, Obata K, Mizushima T, Sakurai J, Kobayashi K, Yamanaka H, Dai Y, Fukuoka T, Sakagami M, Noguchi K. Activation of Src-family kinases in spinal microglia contributes to mechanical hypersensitivity after nerve injury. J Neurosci 2006;26:8680–90.

[41] Kawasaki Y, Zhang L, Cheng JK, Ji RR. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci 2008;28: 5189–94.

[42] Kiguchi N, Kobayashi Y, Kishioka S. Chemokines and cytokines in neuroinflammation leading to neuropathic pain. Curr Opin Pharmacol 2012;12:55–61.

[43] Kwiatkowski K, Piotrowska A, Rojewska E, Makuch W, Jurga A, Slusarczyk J, Trojan E, Basta-Kaim A, Mika J. Beneficial properties of maraviroc on neuropathic pain development and opioid effectiveness in rats. Prog Neuropsychopharmacol Biol Psychiatry 2016;64:68–78.

[44] Larsson A, Carlsson L, Gordh T, Lind AL, Thulin M, Kamali-Moghaddam M. The effects of age and gender on plasma levels of 63 cytokines. J Immunol Methods 2015;425:58–61.

[45] Lin CP, Kang KH, Lin TH, Wu MY, Liou HC, Chuang WJ, Sun WZ, Fu WM. Role of spinal CXCL1 (GROalpha) in opioid tolerance: a human-to-rodent translational study. Anesthesiology 2015;122:666–76.

[46] Lind AL, Emami Khoonsari P, Sjodin M, Katila L, Wetterhall M, Gordh T, Kultima K. Spinal cord stimulation alters protein levels in the cerebrospinal fluid of neuropathic pain patients: a proteomic mass spectrometric analysis. Neuromodulation 2016;19:549–62.

[47] Lundberg M, Eriksson A, Tran B, Assarsson E, Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. Nucleic Acids Res 2011;39:e102.

[48] Lundborg C, Hahn-Zoric M, Biber B, Hansson E. Glial cell line-derived neurotrophic factor is increased in cerebrospinal fluid but decreased in blood during long-term pain. J Neuroimmunol 2010;220:108–13.

[49] Makker PG, Duffy SS, Lees JG, Perera CJ, Tonkin RS, Butovsky O, Park SB, Goldstein D, Moalem-Taylor G. Characterisation of immune and neuroinflammatory changes associated with chemotherapy-induced peripheral neuropathy. PLoS One 2017;12:e0170814.

[50] Mao J. Translational pain research: achievements and challenges. J Pain 2009;10:1001–11.

[51] Mika J, Zychowska M, Popiolek-Barczyk K, Rojewska E, Przewlocka B. Importance of glial activation in neuropathic pain. Eur J Pharmacol 2013; 716:106–19.

[52] Miller RJ, Jung H, Bhangoo SK, White FA. Cytokine and chemokine regulation of sensory neuron function. Handb Exp Pharmacol 2009: 417–49.

[53] Milligan E, Zapata V, Schoeniger D, Chacur M, Green P, Poole S, Martin D, Maier SF, Watkins LR. An initial investigation of spinal mechanisms underlying pain enhancement induced by fractalkine, a neuronally released chemokine. Eur J Neurosci 2005;22:2775–82.

[54] Milligan ED, Zapata V, Chacur M, Schoeniger D, Biedenkapp J, O'Connor KA, Verge GM, Chapman G, Green P, Foster AC, Naeve GS, Maier SF, Watkins LR. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. Eur J Neurosci 2004;20: 2294–302.

[55] Moen A, Lind AL, Thulin M, Kamali-Moghaddam M, Roe C, Gjerstad J, Gordh T. Inflammatory serum protein profiling of patients with lumbar radicular pain one year after disc herniation. Int J Inflam 2016;2016: 3874964.

[56] Mullington JM, Simpson NS, Meier-Ewert HK, Haack M. Sleep loss and inflammation. Best Pract Res Clin Endocrinol Metab 2010;24:775–84.

[57] O'Callaghan JP, Sriram K, Miller DB. Defining "neuroinflammation." Ann N Y Acad Sci 2008;1139:318–30.

[58] O'Hayre M, Salanga CL, Handel TM, Hamel DJ. Emerging concepts and approaches for chemokine-receptor drug discovery. Expert Opin Drug Discov 2010;5:1109–22.

[59] Olausson P, Gerdle B, Ghafouri N, Sjostrom D, Blixt E, Ghafouri B. Protein alterations in women with chronic widespread pain—an explorative proteomic study of the trapezius muscle. Sci Rep 2015;5:11894.

[60] Old EA, Clark AK, Malcangio M. The role of glia in the spinal cord in neuropathic and inflammatory pain. Handb Exp Pharmacol 2015;227: 145–70.

[61] Parisien M, Khoury S, Chabot-Dore AJ, Sotocinal SG, Slade GD, Smith SB, Fillingim RB, Ohrbach R, Greenspan JD, Maixner W, Mogil JS, Belfer I, Diatchenko L. Effect of human genetic variability on gene expression in dorsal root ganglia and association with pain phenotypes. Cell Rep 2017; 19:1940–52.

[62] Pedersen BK. Muscles and their myokines. J Exp Biol 2011;214:337–46.

[63] Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther 2003;306:624–30.

[64] Roche S, Gabelle A, Lehmann S. Clinical proteomics of the cerebrospinal fluid: towards the discovery of new biomarkers. Proteomics Clin Appl 2008;2:428–36.

[65] Russell IJ, Orr MD, Littman B, Vipraio GA, Alboukrek D, Michalek JE, Lopez Y, MacKillip F. Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. Arthritis Rheum 1994;37: 1593–601.

[66] Sato-Takeda M, Ihn H, Ohashi J, Tsuchiya N, Satake M, Arita H, Tamaki K, Hanaoka K, Tokunaga K, Yabe T. The human histocompatibility leukocyte antigen (HLA) haplotype is associated with the onset of postherpetic neuralgia after herpes zoster. PAIN 2004;110:329–36.

[67] Schmitz K, Pickert G, Wijnvoord N, Haussler A, Tegeder I. Dichotomy of CCL21 and CXCR3 in nerve injury-evoked and autoimmunity-evoked hyperalgesia. Brain Behav Immun 2013;32:186–200.

[68] Strong JA, Xie W, Coyle DE, Zhang JM. Microarray analysis of rat sensory ganglia after local inflammation implicates novel cytokines in pain. PLoS One 2012;7:e40779.

[69] Thacker MA, Clark AK, Bishop T, Grist J, Yip PK, Moon LD, Thompson SW, Marchand F, McMahon SB. CCL2 is a key mediator of microglia activation in neuropathic pain states. Eur J Pain 2009;13:263–72.

[70] Themistocleous AC, Ramirez JD, Shillo PR, Lees JG, Selvarajah D, Orengo C, Tesfaye S, Rice AS, Bennett DL. The Pain in Neuropathy Study (PiNS): a cross-sectional observational study determining the somatosensory phenotype of painful and painless diabetic neuropathy. PAIN 2016;157:1132–45.

[71] Tiwari V, Guan Y, Raja SN. Modulating the delicate glial-neuronal interactions in neuropathic pain: promises and potential caveats. Neurosci Biobehav Rev 2014;45C:19–27.

[72] Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology 2008; 70:1630–5.

[73] Vaeroy H, Helle R, Forre O, Kass E, Terenius L. Cerebrospinal fluid levels of beta-endorphin in patients with fibromyalgia (fibrositis syndrome). J Rheumatol 1988;15:1804–6.

[74] Vaeroy H, Helle R, Forre O, Kass E, Terenius L. Elevated CSF levels of substance P and high incidence of Raynaud phenomenon in patients with fibromyalgia: new features for diagnosis. PAIN 1988;32:21–6.

[75] Walker AK, Kavelaars A, Heijnen CJ, Dantzer R. Neuroinflammation and comorbidity of pain and depression. Pharmacol Rev 2014;66:80–101.

[76] Vallejo R, Tilley DM, Vogel L, Benyamin R. The role of glia and the immune system in the development and maintenance of neuropathic pain. Pain Pract 2010;10:167–84.

[77] van Rooijen DE, Roelen DL, Verduijn W, Haasnoot GW, Huygen FJ, Perez RS, Claas FH, Marinus J, van Hilten JJ, van den Maagdenberg AM. Genetic HLA associations in complex regional pain syndrome with and without dystonia. J Pain 2012;13:784–9.

[78] Watkins LR, Milligan ED, Maier SF. Mechanisms of glial activation after nerve injury. In: Basbaum AI, Bushnell MC, editors. Science of pain. Oxford, UK: Elsevier, 2009. pp. 429–33.

[79] Wheelock AM, Wheelock CE. Trials and tribulations of 'omics data analysis: assessing quality of SIMCA-based multivariate models using examples from pulmonary medicine. Mol Biosyst 2013;9: 2589–96.

[80] Zhang J, De Koninck Y. Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. J Neurochem 2006;97: 772–83.

[81] Zhang ZJ, Cao DL, Zhang X, Ji RR, Gao YJ. Chemokine contribution to neuropathic pain: respective induction of CXCL1 and CXCR2 in spinal cord astrocytes and neurons. PAIN 2013;154:2185–97.

[82] Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J, Ji RR. Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. Brain Behav Immun 2007;21:642–651.