Characterization of neuroinflammation and periphery-to-CNS inflammatory cross-talk in patients with disc herniation and degenerative disc disease

Vinko Paladaa, Aisha Siddiqah Ahmedb, Anja Finn³, Svante Bergc, Camilla I. Svenssona, Eva Kosekb,⁎

a Department of Physiology and Pharmacology, Karolinska Institutet, 17177 Stockholm, Sweden
b Department of Clinical Neuroscience, Karolinska Institutet, Department of Neuroradiology, Karolinska University Hospital, 17177 Stockholm, Sweden
³ Stockholm Spine Center, 19489 Upplands Väsby, Sweden

ABSTRACT

The aim of the study was to identify inflammatory cytokines/chemokines associated with neuroinflammation and periphery-to-CNS inflammatory cross-talk in degenerative disc disease (DDD) and lumbar disc herniation (LDH), common causes of low back pain (LBP). A secondary aim was to investigate the associations between cytokines and symptom severity.

Methods: In total, 40 DDD and 40 LDH patients were recruited from a surgical waiting list, as well as 39 healthy controls (HC) and 40 cerebrospinal fluid (CSF) controls. The subjects completed questionnaires and pressure algometry was performed at the lumbar spine and forearm. The CSF, serum and disc tissues were collected during surgery. Inflammatory mediators TNF, INFγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and MCP1 were analysed by immunoassay (Meso Scale Discovery) and quantitative real-time polymerase chain reaction (qPCR) was used for analysis of IL-6, IL-8, MCP1 and TSPO expression in intervertebral discs (IVDs).

Results: In the LDH group, we found elevated IL-8 concentrations in CSF indicating neuroinflammation, while IL-8 and MCP1 concentrations in serum were lower compared to HC. The IVD expression of IL-6, IL-8 and TSPO was lower in LDH patients compared to DDD. LDH patients had a positive correlation between IL-8 concentrations in CSF and serum and IL-8 in CSF was associated with higher pain intensity and increased spinal pressure pain sensitivity. The MCP1 concentration in serum was associated with higher global pain ratings and increased spinal pressure pain sensitivity, while IL-6 serum concentration correlated with the intensity of the neuropathic pain component (leg pain) in LDH patients. No differences were found in cytokine CSF concentrations between DDD patients and CSF controls, but DDD patients had lower IL-8 and MCP1 serum concentrations than HC. In female DDD patients, IL-8 and MCP1 concentrations in serum were associated with increased intensity of back pain.

Conclusion: Our results suggest that neuroinflammation mediated by elevated IL-8 concentrations in CSF and IL-8 mediated periphery-to-CNS inflammatory cross-talk contributes to pain in LDH patients and suggest a link between TSPO expression in discs and low back pain.

1. Introduction

Low back pain (LBP) is a leading cause of working disability worldwide with an estimated lifetime prevalence of up to 84% (Balagué et al., 2012). This common and disabling condition negatively affects the quality of life and presents a substantial economic burden through the high costs of healthcare and decreased working productivity (Dagenais et al., 2010). The most common conditions with LBP are degenerative disease (DDD) and lumbar disc herniation (LDH), both frequently associated with referred and radicular leg pain, respectively (Freynhagen et al., 2008; Zhang et al., 2009; Schäfer et al., 2009).

Although the pathogenesis of DDD and LDH is complex, inflammatory mechanisms have been reported in both conditions. In degenerative discs, nociceptors in annulus fibrosus are sensitized to mechanical stimulation due to loss of weight bearing disc properties and to inflammatory stimulation by released pro-inflammatory substances from intervertebral disc (IVD) cells and infiltrating immune cells (Brisby, 2006; Risbud and Shapiro, 2014). Furthermore, the neurotrophins NGF and BDNF, which are produced by IVD cells, enhance ingrowth of nociceptive fibers and pain in degenerative discs (Freemont
et al., 2002; Purmessur et al., 2008; Krock et al., 2014; Krock et al., 2016). In the patients with disc herniation, disc material leaks outside of the annulus fibrosus into the spinal canal causing pressure on spinal nerve roots and local inflammation which contribute to the pain (Bono et al., 2006; Schoenfeld and Weiner, 2010). Increased levels of pro-inflammatory cytokines IL-1 and TNF were found in human degenerative and herniated disc tissues and suggested to regulate expression of genes encoding for the extracellular matrix-degrading enzymes (Le Maître et al., 2007; Wang et al., 2011).

Several studies showed that peripheral inflammation can lead to increased cytokine production also in CNS. Paw injections of complete Freund’s adjuvant or carrageenan lead to upregulation of IL-1b not only in the inflamed paw (Samad et al., 2001) but also elevated levels in cerebrospinal fluid (CSF) (Samad et al., 2001; Abdelmoaty et al., 2013) and similar was observed for increased TNF concentrations in CSF (Bianchi et al., 2007; Abdelmoaty et al., 2013). Persistent peripheral inflammation was found to stimulate the transfer of pro-inflammatory cytokines from the periphery to the CNS by crossing the blood-brain barrier (BBB) (Gutiérrez et al., 1994; Greter et al., 2005; Mitchell et al., 2008). Furthermore, increased permeability of BBB allows for infiltration of immune cells to the CNS which has been suggested to contribute to neuropathic pain (Ellis and Bennett, 2013; Ji et al., 2014). Infiltrating macrophages differentiate into activated microglia (Zhang et al., 2007, Verma et al., 2015). Activation of glial cells and endothelial cells leads to neuroinflammation with increased production of pro-inflammatory cytokines and chemokines that can modulate pain (Ehrlich et al., 1998; Verma et al., 2006; Ramesh et al., 2013).

Although neuroinflammation was previously linked to different human pain conditions such as neuropathic pain (Kotani et al., 2004; Backonja et al., 2008), rheumatoid arthritis (Lampa et al., 2012) and fibromyalgia (Kadetoff et al., 2012; Bäckryd et al., 2017a), the role of neuroinflammatory mechanisms in LBP is poorly understood. To our knowledge, elevated CSF concentrations of interleukin-8 (IL-8) have been previously reported only in a subgroup of LDH patients compared to the laboratories cutoff values (Brisby et al., 2002). Furthermore, positron emission tomography using a translocator protein (TSPO) ligand to identify neuroinflammation and glia activation was used to assess patients with chronic lower extremity radiculopathy. The authors found increased TSPO binding at the afflicted nerve root and the corresponding part of the spinal cord (Albrecht et al., 2018), which was in accordance with their previous findings with the same ligand indicating cerebral glia activation in patients suffering from non-specific chronic low back pain (Loggia et al., 2015).

We hypothesized that neuroinflammation due to periphery-to-CNS cross-talk through pro-inflammatory mediators could contribute to pain in DDD and LDH patients. The main aim of this study was to characterize CNS neuroinflammatory changes in well-defined cohorts of DDD and LDH patients. We postulated that pro-inflammatory substances involved in neuroimmune communication between blood and CNS would be characterized by a) elevated concentrations in CSF and, b) a positive correlation between the concentrations of that substance in CSF and serum. We chose to compare DDD patients suffering from nociceptive pain with LDH patients with predominantly neuropathic pain. In an exploratory part of the study, we also wanted to assess potential associations of cytokine/chemokine concentrations in CSF and serum with symptom severity. Potential sex differences were examined since higher prevalence of LBP and higher pain intensity were previously reported for female patients with LBP (Bailey, 2009; Fillingim et al., 2009) and sex differences in innate immune reactions have been documented (Levine et al., 2006; Karshikoff et al., 2015, 2016; Doyle and Murphy, 2017; Rosen et al., 2017). Finally, we also characterized the peripheral inflammatory component by examining disc expression of inflammatory cytokines and TSPO, a protein involved in the regulation of inflammation, oxidative stress and cell survival (Gatliff and Campanella, 2015).

2. Materials and methods

2.1. Study participants

The study was conducted on 40 DDD patients (22 women and 18 men, average age 44.4 years, range 27–63 years), 40 LDH patients (12 women and 28 men, average age 41.1 years, range 25–65 years) and 39 healthy controls (24 women and 15 men, average age 49.6 years, range 29–65 years). All patients were recruited from Stockholm Spine Center, Upplands Väsby, Sweden while healthy subjects were recruited by advertisement in the local newspapers. DDD patients were on the waiting list for disc replacement or spinal fusion surgeries whereas LDH patients were on the waiting list for discectomy. The local ethical committee (2011/2036-31/1) approved the study and all patients and healthy subjects signed the written consent forms.

The inclusion criteria for DDD patients were: 25–70 years of age, radiologically confirmed degenerative changes in the lumbar spine and on the waiting list for fusion or disc implants 1–3 levels (L3-S1) at Stockholm Spine Center. To be eligible for inclusion, the pain duration had to be > 1 year and theVAS ratings of average weekly pain intensity > 30 mm on a 100 mm visual analogue scale (VAS). For LDH patients, the inclusion criteria were: 25–70 years of age, radiologically (MR) confirmed lumbar disc herniation (L3-S1) with symptoms of radiculopathy consistent with the radiological findings, absence of Modic changes, maximum of 50% reduction of disc space (to exclude patients with DDD). To be eligible for inclusion the patients pain duration had to be > 1 month with leg pain dominating over back pain, VAS ratings of average weekly back pain < 30 mm and no previous history of significant back pain. The exclusion criteria for both groups of patients were chronic pain due to other reasons such as fibromyalgia, knee or hip osteoarthritis, inflammatory rheumatic diseases or neurologic disorders and previous surgery at investigated levels. Exclusion criteria for healthy controls were chronic pain due to fibromyalgia, knee or hip osteoarthritis, disc degenerative disease, disc herniation, inflammatory rheumatic disease or neurologic disease or VAS ratings of weekly average pain > 20 mm. The HC were pre-screened by telephone and once more on the day of examination. Information regarding medication was collected from all patients. In the DDD group 20 patients were taking analgesics (7 codeine, 4 tramadol, 1 buprenorphine plaster, 8 strong opioids orally), 14 were taking acetaminophen, 3 were on antidepressants, 2 on anticonvulsants and 5 had previously been taking NSAIDs, however the NSAIDs were stopped due to the planned surgical procedure 2 weeks prior to the surgery. In the LDH group 17 patients were taking analgesics (8 codeine, 4 tramadol, 5 strong opioids orally), 17 were taking acetaminophen, 3 were on antidepressants, 6 on anticonvulsants and 10 had previously been taking NSAIDs; the NSAIDs were stopped 2 weeks prior to the surgery. All received 1 g acetaminophen (paracetamol) as premedication before surgery and DDD patients were also given 20 mg oxycodone orally.

Serum samples were obtained from all patients and HC. Additionally, CSF was collected from 40 patients (23 women and 17 men, average age 47.4 years, range 26–73 years) with non-inflammatory neurological symptoms which were used as control group for CSF measurements. These patients had been investigated for headache at the Department of Neurology at Karolinska University Hospital. The routine blood tests, CSF analysis and brain MR showed no signs of inflammatory disease in this cohort. No medications were taken on a regular basis and no analgesics had been used on the day of assessment. None of the headache controls had any painful disorders (except headache). The CSF controls had given their permission that their CSF would be used for research purposes.

2.2. Procedure

2.2.1. Patients

The patients completed the questionnaires and pressure pain sensitivity was determined by pressure algometry, typically within a week.
from surgery. On the day of surgery, before the surgical procedure started, intravenous blood samples were collected from the antecubital vein and lumbar puncture was performed to collect the CSF before general analgesia. Disc tissue was harvested during surgery.

2.2.2. Healthy controls (HC)

The HC were screened by telephone and those who complied with the inclusion criteria were scheduled for assessment with questionnaires, pressure algometry, following the same protocol as the patients and intravenous blood samples were collected from the antecubital vein.

2.3. Questionnaires

Global pain intensity at the day of examination, as well as back pain and leg pain were all scored using 100 mm visual analogue scale (VAS) where 0 indicated “no pain” and 100 indicated “the worst imaginable pain”. Hospital Depression and Anxiety (HAD) Scale was used to access the level of anxiety and depression. The HAD scale is a questionnaire developed for non-psychiatric patients and consists of two subscales (for the anxiety and depression) which have a range from 0 (no anxiety or depression) to 21 (the maximal anxiety or depression) (Bjelland et al., 2002). Sleep quality was examined by The Pittsburgh Sleep Quality Inventory (PSQI) which is a 19-item questionnaire with scores ranging from 0 to 21, where higher scores reflect the worse sleep (Buysse et al., 1989). Information regarding general fatigue was obtained by Multi-dimensional Fatigue Inventory (MFI-20) which is a 20-item questionnaire with the scale from 4 to 20, where higher scores indicate more fatigue (Lundh Hagelin et al., 2007; Ericsson and Mannerkorpi, 2007; Lin et al., 2009). The EQ-5D was used to access the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. There are 3 levels for each EQ-5D scale and the compact scores have a range from 1 (no problems) to −0.59 (the most severe problems) (Wynes et al., 2008; Devlin and Brooks, 2017). Oswestry Disability Index (ODI) was used to determine the disability of the patients with low back pain by calculating the index with the range from 0 (no disability) to 100 (maximal possible disability) (Fairbank et al., 1980).

2.4. Sensory testing protocol

Pressure pain sensitivity was assessed using a calibrated algometer (Somedic Sales AB, Hörby, Sweden) with a flat circular tip area of 1 cm² at the rate of pressure increase of approximately 50 kPa/s (Kosek et al., 1993). Pressure pain thresholds (PPTs) and pressure pain (PP) corresponding to 4/10 (PP4) and 7/10 (PP7) on Borg’s category ratio (CR)-10 scale (Borg, 1982) were assessed at the middle between L4 and S1 and dorsally at the right forearm. To assess PPTs, subjects were asked to press a button as soon as the pressure became painful, three assessments per site were performed and the average of the 3 assessments was calculated and used for further analysis. To assess PP4 or PP7, subjects were asked to press the button when pain reached intensities rated as 4 (moderate to strong pain) (PP4) or 7 (very strong pain) (PP7), respectively, on the Borg’s CR-10 scale, where 0 = no pain and 10 = extremely strong pain (Fridén et al., 2013). Since gender distribution in DDD and LDH groups was not balanced and gender differences in pressure pain sensitivity have been reported, we calculated the relative pressure pain sensitivity for all individuals by calculating a ratio between spine and arm PPT for each individual (e.i., PPTspine/PPTarm) these normalized values are abbreviated as NPPT, NPP4 and NPP7, respectively.

2.5. Intraocular sample collection and storage

Interventricular discs collected from DDD and LDH patients were homogenized by Micro-disemembrator (B. Braun Biotech International, Germany) and dissolved in 2–3 volumes of Trizol reagent (Invitrogen Life Technologies Inc., USA). Total RNA was extracted using the RNeasy MiniKit (Qiagen, USA) following the manufacturer’s instructions. Quantity of RNA was determined using a Nanodrop ND-1000 spectrophotometer (Isogen Life Science, Sweden) and the quality of extracted RNA was assessed using the Experion automated electrophoresis system (BioRad, Sweden). First-strand cDNA were synthesized from 0.5μg of total RNA using the first-strand cDNA Synthesis Kit (Roche, Germany). Quantitative real-time PCR was performed with StepOne Plus System (Applied Biosystems, USA) using TaqMan fast PCR master mix (Applied Biosystems, USA). Specific primers for IL-6 (Hs00985639_m1), IL-8 (Hs00174103_m1), MCP1 (Hs00234140_m1) and TSPO (Hs00559362_m1) were used to detect the target. Ct values were calculated by StepOne Software v2.3 (Applied Biosystems, USA). Relative gene expression was analyzed using 2^-ΔΔCt method and the Ct values were normalized to HPRT1 (Hs00280695_m1) as the reference. We used cDNA from primary human fibroblasts-like synoviocytes as the positive control. The assessment of disc mRNA for IL-6, IL-8 and MCP1 was based on our CSF and serum results. We chose to assess disc TSPO expression as this was recently reported to be increased in inflamed joints in patients suffering from rheumatoid arthritis and was related to increased TSPO ligand binding in these patients (Narayan et al., 2018). As TSPO ligands have been used to detect even subclinical levels of peripheral inflammation (Gent et al., 2014), we included TSPO as a mean to identify even small group differences in disc inflammation.

2.6. Detection of inflammatory cytokines/chemokines by immunoassay

Frozen interventricular discs collected from DDD and LDH patients were analyzed using commercially available tenplex immunoassay kits for TNF, INFg, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and a uniplex for MCP1 (Meso Scale Discovery, Rockville, MD, USA). All samples were randomized before assay procedures. The measurement was performed according to manufacturer’s instructions.

2.7. Quantitative real-time polymerase chain reaction (qPCR)

The concentrations of inflammatory cytokines/chemokines in CSF were analyzed by qPCR with the use of qPCR instruments (StepOnePlus System, Applied Biosystems, USA) using TaqMan fast PCR master mix, primer and probe sets from Applied Biosystems. Specific primer and TaqMan probe sets for IL-6, IL-8, IL-10, TNF, MCP1, IL-1b, INFg, IL-12p70, IL-4, IL-13, IL-2, IL-10 and MCP1 were obtained from Invitrogen Life Technologies Inc., USA and were used in all experiments. Relative gene expression was calculated using the ΔΔCt method. The expression level of each gene was normalized to the expression level of HPRT1 (Hs00280695_m1) as the reference. The expression of each gene was analyzed using the StepOnePlus System (Applied Biosystems, USA) and the data was normalized to the expression level of the reference gene (HPRT1).

2.8. Statistical analysis

The data analysis and visualization was performed by Prism 5.0 (Graphpad Software Inc, USA) and SPSS 24.0 (IBM Corp, USA). Non-parametric Mann-Whitney U test was used for the comparison between the groups. In order to avoid the bias by excluding subjects with low cytokine concentrations from the analysis, we substituted values below limit of quantification (LOQ) with the respective LOQ value when group comparisons were performed. The significance of correlations was determined by Spearman’s rank correlation test (two-tailed) and Fisher’s r-to-z transformation was used to calculate differences between correlation coefficients. The univariate analysis of covariance was applied to observe the effect of age and BMI as covariates. P values < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

Characteristics of DDD patients, LDH patients and healthy controls (HCs) are listed in Table 1. As expected, patient groups had significantly higher VASglobal pain intensities compared to HC group (p < 0.0001). Pain intensities in the back and legs were significantly higher at −80 °C for future analysis.
Table 1. Characteristics of study participants. The values are presented as means, with the indicated minimum and maximum. DDD=Degenerative disc disorder; LDH=Lumbar disc herniation; HC=Healthy controls. VAS = Visual analogue scale for global pain (0–100; 0=no pain; 100=the worst imaginable pain); HAD=Hospital anxiety and depression scale (0–21; 21=the worst anxiety/depression); PSQI=Pittsburg sleep quality inventory (0–21, 21=the worst sleep quality); MFI=Multidimensional fatigue inventory, general fatigue (4–20, the worst disability, 100=maximal possible disability). BMI=Body mass index; Diff=Group difference; NS=not significant (p > 0.05).

|                         | DDD males (n=40) | DDD females(n=22) | LDH males(n=28) | LDH females(n=12) | HC (n=39) | DDD vs HC | LDH vs HC | Diff LDH vs DDD | NS P=0.0184 NS 26.34(24.44–29.26) |
|-------------------------|------------------|-------------------|-----------------|-------------------|-----------|-----------|-----------|----------------|----------------|
| **Age (years)**         | 44.45 (29–63)    | 42.73 (30–63)     | 42.11 (29–63)   | 40.64 (29–63)     | 40.96 (29–63) | NS        | NS        | NS             | 0.37(−0.25–0.8) |
| **BMI (kg/m²)**         | 24.4 (20–31)     | 24.4 (20–31)      | 24.4 (20–31)    | 24.4 (20–31)      | 24.4 (20–31) | NS        | NS        | NS             | 0.279(−0.14–0.85) |
| **VAS global (mm)**     | 46.28 (3–79)     | 47.36 (4–97)      | 47.48 (4–97)    | 47.36 (4–97)      | 47.48 (4–97) | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **VAS back (mm)**       | 31.82 (4–81)     | 26.12 (4–57)      | 25.55 (0–95)    | 26.12 (4–57)      | 26.12 (4–57) | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **VAS leg (mm)**        | 5.538 (0–45)     | 5.338 (0–45)      | 2.059 (0–20)    | 2.059 (0–20)      | 2.059 (0–20) | NS        | NS        | NS             | 0.279(−0.14–0.85) |
| **Anxiety (HAD-A)**     | 6.69 (1–17)      | 6.7 (1–14)        | 6.64 (0–14)     | 6.64 (0–14)       | 6.64 (0–14) | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **Depression (HAD-D)**  | 15.64 (7–20)     | 15.2 (7–20)       | 15.1 (5–20)     | 15.1 (5–20)       | 15.1 (5–20) | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **Disability, ODI**     | 21 (13–37)       | 22.2 (13–37)      | 20.7 (13–37)    | 20.7 (13–37)      | 20.7 (13–37) | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **Hemoglobin (g/dL)**   | 15.6±0.1         | 15.5±0.1          | 15.3±0.1        | 15.3±0.1          | 15.3±0.1    | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **Hematocrit (%)**      | 45.0±0.6         | 45.0±0.6          | 44.8±0.6        | 44.8±0.6          | 44.8±0.6    | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **Mean corpuscular volume (fL)** | 87.6±0.3    | 87.6±0.3          | 87.6±0.3        | 87.6±0.3          | 87.6±0.3    | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **Platelets (10⁹/L)**   | 244±23           | 244±23            | 244±23          | 244±23            | 244±23      | NS        | NS        | NS             | 0.30(−0.30–0.9) |
| **Leukocytes (10⁹/L)**  | 8.1±0.3          | 8.1±0.3           | 8.1±0.3         | 8.1±0.3           | 8.1±0.3     | NS        | NS        | NS             | 0.30(−0.30–0.9) |

The concentrations in CSF were below the limit of quantification (LOQ) for all substances, with the exception of TNF (LOQ = 0.62 pg/ml), INFg (LOQ = 2.74 pg/ml), IL-1b (LOQ = 0.98 pg/ml), IL-2 (LOQ = 0.70 pg/ml), IL-4 (LOQ = 0.42 pg/ml), IL-10 (LOQ = 0.16 pg/ml), IL-12p70 (LOQ = 0.80 pg/ml) and IL-13 (LOQ = 4.14 pg/ml).

3.2. Sensory changes in DDD and LDH patients

The most prominent difference between the two groups of patients was statistically significant reduction in spinal pressure pain threshold (PPT) only in DDD patients (p = 0.0263, Table 2) while there was no reduction in spinal PPT for LDH patients compared to HC. The increased pressure pain sensitivity was even more pronounced for the suprathreshold pain stimuli PP4 (p = 0.014) and PP7 (p = 0.0051) in patients with DDD compared to HC. Furthermore, while not showing decrease in spinal PPT, LDH patients had a characteristic decrease of PPT in the arm compared to HC group (p = 0.0196) which was not present for PP4 and PP7. The normalized pressure pain threshold (NPPT) was reduced in both groups of patients compared to HC, indicating a relative increase in spinal tenderness. However, normalized pressure pain thresholds for the suprathreshold pain stimuli were reduced only in DDD patients versus controls PP4 (p = 0.046) and PP7 (p = 0.0065).

3.3. Group differences in inflammatory cytokines and chemokines

3.3.1. Cerebrospinal fluid

A significantly higher concentration of IL-8 was detected in the CSF of LDH patients compared to DDD patients (p = 0.0024) and CSF controls (p = 0.0071; Fig. 1, Table 3), but no gender differences were found. There were no statistically significant differences between the groups and genders for the CSF concentrations of IL-6 and MCP-1 (Table 3). BMI and age had no significant effect on CSF concentrations of IL-8, IL-6 and MCP1 between the groups as measured by univariate analyses of covariance. There were no significant differences in the IL-8, IL-6 and MCP1 levels in CSF between male and female CSF controls. The concentrations in CSF were below the limit of quantification (LOQ) in all subjects for the following substances: TNF (LOQ = 0.62 pg/ml), INFg (LOQ = 2.74 pg/ml), IL-1b (LOQ = 0.98 pg/ml), IL-2 (LOQ = 0.70 pg/ml), IL-4 (LOQ = 0.42 pg/ml), IL-10 (LOQ = 0.16 pg/ml), IL-12p70 (LOQ = 0.80 pg/ml) and IL-13 (LOQ = 4.14 pg/ml).

3.3.2. Serum

The analysis of serum samples revealed significantly lower concentrations of IL-8 in DDD patients (p < 0.0001) and LDH patients (p = 0.0013) compared to HC group while there was no difference between the patient groups (Fig. 1, Table 3). Furthermore, male DDD patients had elevated serum concentrations of IL-8 compared to DDD patient groups compared to healthy subjects. Also, as expected LDH patients had significantly higher VAS ratings for pain intensity in the legs compared to DDD patients (p = 0.0079). Furthermore, patients with DDD and LDH had higher ratings of anxiety and depression (HADS scores). Fifteen out of 40 DDD patients (37.5%) and 14 out of 40 LDH patients (35%) had anxiety HADS higher than the proposed cut-off value of 8 (Bjelland et al., 2002). Additionally, 16 DDD patients (40%) and 13 LDH patients (32.5%) had ratings of depression HADS > 8 indicating that the mood changes were frequent in both patient groups. On a scale from 4 to 20 (MFI-20), DDD and LDH patients rated the general fatigue as 15.6 and 15.1 respectively, pointing to the presence of severe fatigue which was not seen in HCs and which is in accordance with the significantly reduced quality of sleep (PSQI, p < 0.0001) in both groups. In general, DDD and LDH seemed to have a negative impact on the quality of life (EQ-5D, p < 0.0001) and the ability to perform usual activities in the patients compared to HCs. Both patient groups developed moderate disability due to LBP (ODI scores between 21 and 40). In our study, HCs were older than DDD and LDH patients (p < 0.05) and LDH patients had elevated body mass index (BMI) compared to HCs (p = 0.0184). Regarding gender differences, we observed increased pain intensity in DDD female patients versus DDD males (VAS global, p = 0.0201). Also, DDD male patients had higher BMI compared to females (p < 0.05).
Table 2
Comparison of pressure pain sensitivity. The values are presented as means, together with standard deviation (SD); mean (SD). DDD = Degenerative disc disorder; LDH = Lumbar disc herniation; HC = Healthy controls. PPT = Pressure pain threshold; PP4 = Pressure pain corresponding to pain intensity of 4 at Borg scale; PP7 = Pressure pain corresponding to pain intensity of 7 at Borg scale; NPP4 = normalized pressure pain threshold (PPT spine/PPT arm); NPP7 = normalized pressure pain corresponding to pain intensity of 4 at Borg scale; NS = not significant (p > 0.05).

|                  | PPT Arm (kPa) | PPT4 Arm (kPa) | PPT7 Arm (kPa) | PPT Spine (kPa) | PPT7 Spine (kPa) | NPP4 | NPP7 |
|------------------|--------------|---------------|---------------|----------------|-----------------|------|------|
| DDD (n = 40)     | 336.3 (146.4)| 474.9 (193.8) | 580.5 (219.6) | 325.9 (169.4)  | 459.6 (183.2)   | NS   | NS   |
| LDH (n = 40)     | 456.6 (311.8)| 549.2 (249.9) | 615.0 (203.1) | 407.2 (189.1)  | 585.8 (280.8)   | NS   | NS   |
| HC (n = 39)      | 315 (161.8)  | 485.9 (157.7) | 623.5 (168.8) | 412.6 (169.1)  | 567.5 (18.6)    | P = 0.0263 | NS   |
| DDD vs HC Diff   | NS           | NS            | NS            | NS             | NS              | P = 0.0015 | NS   |
| LDH vs HC Diff   | P = 0.0196   | NS            | NS            | NS             | NS              | NS   | NS   |
| DDD vs LDH Diff  | NS           | NS            | NS            | NS             | NS              | NS   | NS   |
|                  | mean (SD)    | mean (SD)     | mean (SD)     | mean (SD)      | mean (SD)       |      |      |
| DDD              | 336.3 (146.4)| 474.9 (193.8) | 580.5 (219.6) | 325.9 (169.4)  | 459.6 (183.2)   |      |      |
| LDH              | 456.6 (311.8)| 549.2 (249.9) | 615.0 (203.1) | 407.2 (189.1)  | 585.8 (280.8)   |      |      |
| HC               | 315 (161.8)  | 485.9 (157.7) | 623.5 (168.8) | 412.6 (169.1)  | 567.5 (18.6)    |      |      |
|                  | mean (SD)    | mean (SD)     | mean (SD)     | mean (SD)      | mean (SD)       |      |      |
| DDD vs HC Diff   | NS           | NS            | NS            | NS             | NS              |      |      |
| LDH vs HC Diff   | P = 0.0196   | NS            | NS            | NS             | NS              |      |      |
| DDD vs LDH Diff  | NS           | NS            | NS            | NS             | NS              |      |      |

3.4. Correlation of cytokine/chemokine levels between CSF and serum

There was a significant positive correlation between CSF and serum concentrations of IL-8 in LDH patients (r = 0.489; p = 0.002; Fig. 2a), but not in DDD patients. Additionally, we found statistically significant positive correlation of MCP1 between CSF and serum in DDD patients (r = 0.332; p = 0.037; Fig. 2b), but not in the LDH group. No significant correlations were found between IL-6 concentrations in CSF and serum. There were no statistically significant gender differences in the correlation coefficients.

3.5. Association of cytokine/chemokine levels with pain and clinical symptoms

In the exploratory part of the study, we examined the association of cytokine/chemokine concentrations in CSF and serum with the global pain intensity (VAS global), pain intensities in back (VAS back) and leg (VAS leg), spinal pressure pain threshold (PPT) and disability (ODI) (Table 4). Based on our previous findings of profound sex differences in the associations between cytokine concentrations and pain modulation (Karshikoff et al., 2015; Kosek et al., 2018) we also included separate analysis of male and female patients (Table 4). Analyzing LDH male and female patients together, a positive correlation was found between IL-6 in serum and leg pain intensity (r = 0.380; p = 0.017) and MCP1 in serum was associated with increased pressure pain sensitivity at the spine (negatively correlated with spinal PPTs) (r = −0.338; p = 0.044). In addition, in male LDH patients IL-8 in CSF was associated with global pain intensity (r = 0.395; p = 0.038) as well as increased pressure pain sensitivity at the spine (r = −0.546; p = 0.004) whereas MCP1 in serum was associated with global pain intensity (r = 0.515; p = 0.006). Female DDD patients had a positive correlation between back pain and IL-8 in serum (r = 0.462; p = 0.035) as well as MCP1 in serum (r = 0.488; p = 0.021).

3.6. Gene expression analysis of cytokine/chemokine levels in the intervertebral discs and their associations to back and leg pain

The gene expression of IL-6, IL-8 and TSPO in IVDs was significantly higher in DDD patients compared to LDH (Fig. 3). There were no significant group differences in IVD expression of MCP1. No gender differences were found for any of the analysed substances, in either group (data not shown). We did not find an association between the gene expression of IL-6, IL-8 and MCP1 in IVDs and the level of respective cytokines in serum and CSF (p > 0.05). There was a significantly positive correlation between IL-6 and IL-8 IVD expression in both patient groups (Table 5). The IVD expression of MCP-1 was significantly correlated to IL-6 both in DDD and LDH patients while it correlated to IL-8...
Table 3
Concentrations of inflammatory cytokines and chemokines in CSF and serum (pg/ml). Data are presented as the medians with 25% and 75% percentile values. DDD = Degenerative disc disorder; LDH = Lumbar disc herniation; HC = Healthy controls; NS = Not significant; LOQ = Limit of quantification. Values below LOQ were set as equal to LOQ and the number of values substituted with LOQ are presented in the table as (<LOQ=).

| CSF (pg/ml) | DDD (n=49) | LDH (n=40) | HC controls (n=40) | DDD vs HC controls DIFF | LDH vs HC controls DIFF | DDD vs LDH DIFF | DDD men (n=18) | DDD women (n=31) | LDH men (n=22) | LDH women (n=20) | HC men (n=17) | HC women (n=23) | LDH men vs HC controls DIFF | LDH women vs HC controls DIFF | DDD men vs LDH men DIFF | DDD women vs LDH women DIFF |
|-------------|-------------|-------------|-------------------|-------------------------|-------------------------|-------------------|--------------|----------------|-------------|----------------|-------------|----------------|---------------------------|---------------------------|-------------------------|-------------------------|
| TNF-α       | 0.79        | 0.89        | 0.8               | NS                      | NS                      | NS                | 0.95         | 0.98          | 0.57        | 0.0            | 0.94         | 0.76          | NS                       | NS                       | NS                      | NS                      |
| (LOQ=0.75)  | (>LOQ=0)    | (>LOQ=0)    | (>LOQ=0)          | (>LOQ=0)               | (>LOQ=0)               | (>LOQ=0)         | (>LOQ=0)    | (>LOQ=0)     | (>LOQ=0)   | (>LOQ=0)     | (>LOQ=0)   | (>LOQ=0)     | (>LOQ=0)                  | (>LOQ=0)                  | (>LOQ=0)               | (>LOQ=0)               |
| IL-6        | 26.54       | 29.34       | 26.12             | NS                      | NS                      | NS                | 29.66        | 29.64         | 37.2        | 31.64         | 31.65       | 29.75         | NS                       | NS                       | NS                      | NS                      |
| (LOQ=6.98)  | (>LOQ=0)    | (>LOQ=0)    | (>LOQ=0)          | (>LOQ=0)               | (>LOQ=0)               | (>LOQ=0)         | (>LOQ=0)    | (>LOQ=0)     | (>LOQ=0)   | (>LOQ=0)     | (>LOQ=0)   | (>LOQ=0)     | (>LOQ=0)                  | (>LOQ=0)                  | (>LOQ=0)               | (>LOQ=0)               |
| MCP1        | 315.3       | 325.4       | 340.4             | NS                      | NS                      | NS                | 399.4        | 309.5         | 393.1       | 306.7         | 375.2       | 315.5         | NS                       | NS                       | NS                      | NS                      |
| (LOQ=0.11)  | (>LOQ=0)    | (>LOQ=0)    | (>LOQ=0)          | (>LOQ=0)               | (>LOQ=0)               | (>LOQ=0)         | (>LOQ=0)    | (>LOQ=0)     | (>LOQ=0)   | (>LOQ=0)     | (>LOQ=0)   | (>LOQ=0)     | (>LOQ=0)                  | (>LOQ=0)                  | (>LOQ=0)               | (>LOQ=0)               |

Fig. 2. Correlation of cytokine/chemokine concentrations between CSF and serum. Two-tailed Spearman’s rank correlation test. A) Correlation between IL-8 concentrations in CSF and serum from LDH patients (r = 0.489; p = 0.002). B) Correlation between MCP1 concentrations in CSF and serum from DDD patients (r = 0.332; p = 0.037). LDH = Lumbar disc herniation; DDD = Degenerative disc disorder. Note: An outlier in Fig. 2A is not presented on the plot.
expression only in LDH patients. TSPO expression showed significant negative correlation to MCP1 and IL-6 gene expression, respectively, in LDH but not in DDD patients (Table 5). IVD expression of TSPO was associated with back pain intensity in LDH (r=0.593; p=0.001), but not in DDD patients, with a significant group difference in correlation coefficients (p=0.040). Furthermore, the positive correlation between TSPO expression and back pain in LDH patients was significant also when the genders were analysed separately, i.e., women (r=0.857; p=0.014) and men (r=0.474; p=0.040). No other statistically significant correlations were found between VAS ratings of back or leg pain and IVD expression.

4. Discussion

4.1. Main findings

To our knowledge, this is the first study comparing the concentrations of inflammatory cytokines in the CSF of LDH and DDD patients and controls and also the first study to report the correlations between CSF and serum levels. We found higher IL-8 levels in CSF of LDH patients compared to DDD patients and controls indicating neuroinflammation. In accordance with our a priori criteria, we identified IL-8 as a potential candidate substance for blood borne, neuroimmune cross-talk in LDH patients, as IL-8 concentrations in CSF were higher in patients than controls and a positive correlation between IL-8 concentrations in CSF and serum were found. Furthermore, in male LDH patients, IL-8 concentration in CSF was associated with higher global pain ratings and increased spinal pain sensitivity, indicating that IL-8 related mechanisms might be involved in the pathophysiology of LDH pain, at least in men. We found indications that MCP1 concentration in serum could be associated with the nociceptive pain component, as it was related to increased pain in both groups and spinal pain sensitivity in LDH patients, while IL-6 in serum is related to the neuropathic pain component in LDH patients. Contrary to LDH, we did not find any evidence for neuroinflammation in the DDD cohort, but the positive correlation between MCP1 concentrations in the CSF and serum

| Table 4 Correlation of cytokine/chemokine concentrations with pain and clinical symptoms. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|   | CSF IL-8 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   | DDD | DDD males | DDD females | LDH | LDH males | LDH females | DDD | DDD males | DDD females | LDH | LDH males | LDH females |
| VAS global (mm) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| VAS back (mm) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| VAS leg (mm) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Spine PPT | r=0.546 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| ODI | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
|   | CSF MCP-1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   | DDD | DDD males | DDD females | LDH | LDH males | LDH females | DDD | DDD males | DDD females | LDH | LDH males | LDH females |
| VAS global (mm) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| VAS back (mm) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| VAS leg (mm) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Spine PPT | r=0.489 | NS | NS | NS | NS | NS | NS | NS | NS |
| ODI | NS | NS | NS | NS | NS | NS | NS | NS | NS |

Spearman correlation coefficients (r) and p-values are shown. VAS global = global pain intensity on a day of examination on a visual analogue scale (high scores reflect more pain), VAS back = pain intensity in the back, VAS leg = pain intensity in the leg, Spine PPT = spinal pressure pain threshold, ODI = Oswestry Disability Index (0 = no disability, 100 = maximal possible disability). NS = not significant (P > 0.05).
indicated that MCP1 could be relevant for neuroimmune communication across the BBB. The association between IL-8 and MCP1 concentrations in serum with the intensity of back pain in female DDD patients and higher IL-6, IL-8 and TSPO IVD expression in DDD compared to LDH patients indicate that peripheral inflammatory mechanisms are relevant in DDD.

4.2. Group differences in cytokine/chemokine concentrations and expression

Neuroinflammation, indicated as elevated IL-8 concentrations in CSF, has previously been reported in patients suffering from nociceptive pain (Kadetoff et al., 2012; Bäckryd et al., 2017a) as well as nociceptive pain (Lundborg et al., 2010), although inconsistent findings have been reported regarding neuropathic pain (Backonja et al., 2008; Bäckryd et al., 2017b). Our findings of elevated IL-8 concentrations in CSF of LDH group are in accordance with Brisby et al. (2002) who reported higher IL-8 concentrations in CSF from a subgroup of LDH patients, compared to the laboratories cut-off values. Furthermore, we found lower IL-8 and MCP1 concentrations in serum from LDH and DDD patients compared to controls, while no group differences were found for IL-6. To our knowledge, only two previous studies have directly compared serum cytokines in LBP patients to HC. Wang et al. (2016) found higher IL-6 and IL-8 concentrations in serum from LDH patients while Weber et al. (2016) reported higher IL-6, but not IL-8, in a cohort consisting of patients diagnosed with LDH, DDD or spinal stenosis; MCP1 was not assessed. Romero-Sanchez et al. (2011) found that MCP1 was lower in patients suffering from mechanical low back pain (likely LDH/DDD) compared to ankylosing spondylitis and although no statistical comparisons were made between the mechanical low back pain patients and healthy controls, the figures indicate either no significant group differences or higher serum IL-6 and lower serum IL-8 and MCP1 in the back pain patients compared to HC. Finally, Pedersen et al. (2015) reported higher serum IL-6 and IL-8 concentrations in LDH patients with severe pain compared to mild pain at 12 months, while Wang et al. (2016) found elevated serum IL-6, but not IL-8, in LDH patients with severe compared to mild radicular pain. Therefore, the results regarding serum concentrations of cytokines and their relationship to pain in chronic LBP cohorts are inconsistent and further

| Table 5 | Correlation of cytokine/chemokine and TSPO gene expression in IVDs. Spearman correlation coefficients (r) and p-values are shown. DDD = Degenerative disc disorder, LDH = Lumbar disc herniation. NS = Not significant (p > 0.05). |
| IVD Gene Expression | IL-6 | IL-8 | MCP1 | TSPO |
|---|---|---|---|---|
| DDD IL-6 | r = 0.568; p = 0.003 | r = 0.691; p = 0.00013 | NS | NS |
| IL-8 | r = 0.568; p = 0.003 | NS | NS | NS |
| MCP1 | r = 0.691; p = 0.00013 | NS | NS | NS |
| TSPO | NS | NS | NS | NS |
| LDH IL-6 | r = 0.472; p = 0.02 | r = 0.772; p = 0.0001 | r = −0.62; p = 0.001 | NS |
| IL-8 | r = 0.472; p = 0.02 | r = 0.422; p = 0.035 | NS | NS |
| MCP1 | r = 0.772; p = 0.0001 | r = 0.422; p = 0.035 | r = −0.61; p = 0.001 | NS |
| TSPO | r = −0.62; p = 0.001 | NS | r = −0.61; p = 0.001 | P = 0.001 |
studies are needed regarding the potential use of serum samples as biomarkers of chronic LBP. Regarding our study, we cannot exclude that the reduced serum concentrations of MCP1 and IL-8 may be an effect of pre-medication before the surgical procedure. Finally, our gene expression analysis of inflammatory mediators in IVDs revealed that IVD cells from DDD patients had significantly higher expression of IL-6, IL-8 and TSPO compared to LDH patients. The results are in accordance with reports of higher levels of IL-6 (Burke et al., 2002) and IL-8 (Burke et al., 2002; Lee et al., 2009; Sadowska et al., 2018) in the discs of DDD compared to LDH patients. Also, previously it was reported that expression of IL-6 and IL-8 was elevated in nucleus pulposus cells from DDD patients compared to postmortem controls (Phillips et al., 2013) while annulus fibrosus samples from LBP patients had elevated IL-8 expression compared to controls with scoliosis (Zhang et al., 2016). Together, these results indicate an elevated IVD production of IL-6 and IL-8 in patients with DDD.

Despite the fact that TSPO is recognized as an inflammatory marker (Loggia et al., 2015; Albrecht et al., 2018; Narayan et al., 2018), to our knowledge, TSPO expression in IVDs has not been previously assessed. TSPO is highly expressed in activated macrophages (Rupprecht et al., 2010; Narayan et al., 2018) and previous studies have documented macrophage infiltrations both in degenerative (Kawaguchi et al., 2001; Shamji et al., 2010; Nakazawa et al., 2018) and herniated human IVDs (Kawaguchi et al., 2001; Koike et al., 2003; Shamji et al., 2010). However, recent studies indicate that contrary to previous findings in rodents, TSPO in humans is mainly expressed by the reparative, anti-inflammatory type of macrophages (M2), whereas TSPO expression is down-regulated in activated pro-inflammatory (M1) macrophages (Narayan et al., 2018; Owen et al., 2017). We hypothesize that TSPO was expressed by the M2 macrophages in the LDH group, explaining the negative association with the expression of pro-inflammatory cytokines, while other cell types were responsible for the elevated TSPO expression in the DDD patients. TSPO expression was 3 times higher in DDD than LDH patients, an interesting finding as TSPO has also been implicated in mitochondrial function and redox-homeostasis, influencing the production of reactive oxygen species (ROS) (Gatilff and Campanella, 2015). As ROS has been linked to the process of disc degeneration (Feng et al., 2017), future studies addressing the potential mechanistic role of TSPO in disc degeneration are much needed.

4.3. Neuroimmune cross-talk

The neuroimmune interface is important for the development and maintenance of chronic pain (Grace et al., 2014). The bidirectional communication between peripheral tissues and the CNS is not only mediated by the nervous system, there is also a crosstalk within the immune system. The later involves lymphocytes, monocytes/macrophages and mast cells in the peripheral tissues and blood (Littlejohn, 2015; Walsh et al., 2015) as well as endothelial cells and glia in the CNS (Kronfol and Remick, 2000; Zhang et al., 2007; Ji et al., 2013; Xanthos and Sandkühler, 2014; Wu et al., 2017). Signal transmission between the immune competent cells relies to a large extent on diffusible molecules including cytokines/chemokines (Kronfol and Remick, 2000; Franco et al., 2007; Saab and Hains, 2009; Verma et al., 2015). The cytokines may have a direct impact on the CNS as cytokine releasing inflammatory cells invade dorsal root ganglia and glia cells become activated to release cytokines (Schaible, 2014) thus contributing to neuroinflammation, pain and hyperalgesia (Zhang et al., 2007; Grace et al., 2014; Verma et al., 2015). The IL-8 and MCP1 are potent chemokines which can induce an infiltration of blood cells through the BBB (Hammond et al., 1995; Yao and Tsirka, 2014) and MCP1 has been shown to promote the proliferation of the infiltrating cells and their differentiation into activated microglia (Zhang et al., 2007; Verma et al., 2015). In addition, MCP1 is also known to increase the BBB permeability by binding to MCP1 receptors (Hayashida et al., 2001). Activated glia are the main source of IL-8 and MCP1 in the brain (Ehrlich et al., 1998; Koyama et al., 2013), although activated brain endothelial cells forming part of the BBB also produce MCP1 and IL-8 (Wu et al., 2017). Therefore, our finding of positive correlations between IL-8 concentrations in CSF and serum and elevated IL-8 concentrations in CSF of LDH patients indicated that neuroimmune cross-talk mediated by IL-8 might contribute to the neuroinflammation in LDH patients. In the DDD group, a positive correlation was found between MCP1 concentration in CSF and serum which is in accordance with our previous analysis of patients suffering from knee osteoarthritis (OA) (Kosek et al., 2018). Thus, despite normal MCP1 concentrations in CSF from our DDD patients, the results still indicate that MCP1 could be relevant for neuroimmune communication across the BBB.

We found no association between the gene expression of IL-8 or MCP1 in the disc and serum or CSF concentrations of these substances, also IL-8 concentrations in CSF were higher in LDH compared to DDD while the opposite was true regarding disc IL-8 expression. Therefore, we found no indirect evidence of neuroimmune disc-to-CNS cross-talk involving these substances, although we recognize that gene expression is a very crude measure. Also, our data do not support that the elevated CSF IL-8 concentrations in LDH patients could be explained by leakage from the herniated disc since then elevated concentrations of IL-6 and MCP1 in CSF would also be expected, which was not the case.

4.4. Correlation between cytokines, TSPO and symptoms

The relevance of neuroinflammation in LDH was further supported by a positive correlation between IL-8 concentrations in CSF and increased pain and hyperalgesia in male LDH patients. In accordance with previous reports of IL-6 levels in serum being related to pain intensity and persistence of pain following surgery in LDH patients (Pedersen et al., 2015; Moen et al., 2016; Schistad et al., 2014; Wang et al., 2016), we found a positive association between IL-6 in serum and the neuro-pathic (leg) pain intensity in LDH patients. In addition, male LDH patients’ MCP1 concentrations in serum were associated with higher ratings of global pain and increased spinal pressure pain sensitivity, indicating that MCP1 most likely contributes to the nociceptive pain component, hypothetically by stimulating monocyte infiltration to herniated discs. This is supported by the previous report of MCP1 regulated infiltration of immune cells in the rabbit model for disc herniation (Yoshida et al., 2005). In line with this, Moen et al. (2016) reported upregulated MCP1 in serum one year following surgery in LDH patients with persistent pain compared to patients who recovered. Finally, in female DDD patients, IL-8 and MCP1 in serum both correlated with the intensity of back pain. The female DDD patients in our study also had significantly higher pain intensity than DDD males which is in line with the overall higher prevalence of LBP and increased pain intensities in women compared to men (Bailey, 2009; Fillingim et al., 2009). The results are in agreement with our findings in OA patients, where female patients rated higher pain intensity than males and IL-8 in synovial fluid was associated with pain only in women (Kosek et al., 2018). The results indicate gender specific effects of IL-8 and MCP1 in chronic pain, in line with reports of gender differences in IL-8 related mechanisms following immune activation in healthy subjects (Karshikoff et al., 2015, 2016) and profound gender differences regarding the effects of IL-8 and MCP1 in knee OA patients (Kosek et al., 2018). Interestingly, we found a positive association between TSPO IVD expression and ratings of back pain in the LDH group. The fact that this was significant in both genders when analysed independently and the very strong correlation seen in the female patients (r = 0.857) indicate that this was a robust finding.

4.5. Pain and pain sensitivity

To our knowledge, this is the first study that compared pressure pain sensitivity in LBP depending whether the pain originates from degenerative or herniated discs. Pressure algometry identified lower
normalized spinal pressure pain thresholds in both patient groups, indicating a relative increase in spinal tenderness, which was expected since palpation is routinely used during clinical examination to indicate afflicted segments. However, despite the fact that no statistically significant differences were seen between the patient groups, only DDD patients had significantly decreased absolute PPTs and increased sensitivity to spinal suprathreshold pressure pain (PP4, PP7) when compared to HC. The finding is in agreement with the notion that pain in DDD is triggered by peripheral nociceptive mechanisms within degenerative discs and is in line with findings in other patient groups suffering from pain due to degenerative changes in joints, such as knee OA (Graven-Nielsen et al., 2012).

4.6. Limitations

Our study has several limitations. Healthy subjects were somewhat older than DDD and LDH patient groups (average 5 and 8 years, respectively), and although the age difference is small, it could potentially influence the results. Also, for ethical reasons, we had to use an additional CSF control group of patients with recurring headaches which were not truly healthy subjects and no control disc tissue was analyzed. Thus, the findings regarding group differences between patients and controls in our study need to be confirmed in future studies, except for the finding of higher IL-8 in the CSF of LDH patients which has been reported previously. The analysis was restricted to a few cytokines as many fell below the detection level of our method and the cross-sectional nature of the study does not permit any conclusions regarding causality. The type-1 error might be increased in the absence of a multiple comparison correction. Although we could identify substances which satisfied our criteria for potential involvement in the periphery-to-CNS cross-talk, the actual proof of their role in this cross-talk awaits to be confirmed in mechanistic studies. Finally, the analysis of associations between cytokines and symptoms is to be regarded as purely exploratory.

4.7. Conclusions

Overall, our findings suggest that IL-8, and possibly also MCP1, may be involved in periphery-to-CNS cross-talk and an important contribution of IL-8 mediated neuroinflammation to pain and pain sensitivity in LDH patients. In addition, IVD expression of TSPO was related to higher back pain intensity and reduced IVD expression of pro-inflammatory cytokines in the LDH group, while DDD patients had higher TSPO expression than LDH, suggesting a role of TSPO in the pathophysiology of disc degeneration and low back pain.

Acknowledgments

The authors would like to express their deep gratitude to Tycho Tullberg, CEO at Spine Center at the time of patient inclusion for generous support and valuable advice regarding study design and the surgeons at Stockholm Spine Center for patient recruitment and sample harvesting. We wish to thank the anesthesiologists Mats Nyhlén and Terry Judkins for performing the lumbar punctures. We thank Associate Professor Magnus Andersson, Department of Clinical Neuroscience and Department of Neurology, Karolinska University Hospital for providing CSF control samples. Furthermore, we thank Carola Skärvinge and Anna Arvidsson at Stockholm Spine Center for excellent logistic assistance and for organizing the database and Azar Baharpoor, Department of Physiology and Pharmacology, Karolinska Institutet for support and laboratory assistance. We thank Stockholm Spine Center for providing research facilities for the study.

Funding

The study has received funding from Stockholm County Council (EK), Swedish research Council (K2013-52X-22199-01-3 for EK and 542-2013-8373 for CIS), Knut and Alice Wallenberg Foundation (CIS), Swedish Rheumatism Association (ASA), King Gustav V Foundation Sweden (ASA) and Eli Lilly. The research was also funded from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement no.602919 and from a donation from the Lundblad family. The funding sources had no influence on study design or scientific content of this manuscript.

Conflict of interest

The authors declare that no competing interests exist.

References

Abdelmoaty, S., Wigerblad, G., Bas, D.B., Codegelli, S., Fernandez-Zafra, T., El-Awaydi, El-S., Moustafa, Y., Abdelhamid, Ael-D., Brodin, E., Svensson, C.I., 2013. Spinal action of lipoxin A4 and 17β-resolvin D1 attenuate inflammation-induced mechanical hypersensitivity and spinal TNF release. PLoS One 8, e75543.

Abrecht, D., Ahmed, S., Kettner, N., Borra, R., Cohen-Adad, J., Deng, H., Houle, T., Opalacz, A., Roth, S., Melo, M.V., Chen, L., Mao, J., Hooker, J., Loggia, M.L., Zhang, Y., 2018. Neuroinflammation of the spinal cord and nerve roots in chronic radicular pain. Pain In press.

Backonja, M.M., Coe, C.L., Muller, D.A., Schell, K., 2008. Altered cytokine levels in the blood and cerebrospinal fluid of chronic pain patients. J. Neuroimmunol. 195, 157–163.

Bäckryd, E., Tanum, L., Lind, A.L., Larsson, A., Gordh, T., 2017a. 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. 10, 515–525.

Bäckryd, E., Lind, A.L., Thulin, M., Larsson, A., Gerdle, B., Gordh, T., 2017b. 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 158, 2487–2495.

Bailey, A., 2009. Risk factors for low back pain in women: still more questions to be answered. Menopause 16, 3–4.

Balagué, F., Mannion, A.F., Pellissier, F., Cedraschi, C., 2012. Non-specific low back pain. Lancet 379, 482–491.

Bianchi, M., Martucci, C., Ferrario, P., Franchi, S., Sacerdoti, P., 2007. Increased tumor necrosis factor-alpha and prostaglandin E2 concentrations in the cerebrospinal fluid of rats with inflammatory hyperalgesia: the effects of analgesic drugs. Anesth. Analg. 104, 949–954.

Bjelland, I., Dahl, A., Haug, T.T., Neckelmann, D., 2002. The validity of the hospital anxiety and depression scale. an updated literature review. J. Psychosom. Res. 52, 69–77.

Bono, C.M., Wninski, R., Garfin, S.R., 2006. Lumbar disc herniations. In: Herkowitz, H.N., Garfin, S.R., Eismont, F.J., Bell, G.R., Balderston, R.A. (Eds.), The Spine, 5th ed. Saunders, Philadelphia, PA.

Borg, G.A., 1982. Psychophysical bases of perceived exertion. Med. Sci. Sports Exerc. 14, 377–381.

Brisby, H., 2006. Pathology and possible mechanisms of nervous system response to disc degeneration. J. Bone Joint Surg. Am. 88, 68–71.

Brischy, H., Omlarker, K., Larsson, K., Natt, M., Rydevik, B., 2002. Proinflammatory cytokines in cerebrospinal fluid and serum in patients with disc herniation and sciatica. Eur. Spine J. 11, 62–66.

Burke, J.G., Watson, R.W., McCormack, D., Dowling, F.E., Walsh, M.G., Fitzpatrick, J.M., 2002. Intervertebral discs which cause low back pain secrete high levels of pro-inflammatory mediators. J. Bone Joint Surg. Br. 84, 196–201.

Buyse, D., Reynolds, I., Monk, C., Kupfer, D., 1989. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213.

Dagenais, S., Tricco, A.C., Haldeman, S., 2010. Synthesis of recommendations for the assessment and management of low back pain. Curr. Opin. Rheumatol. 22, 157–163.

Deb, M., Wijeysundera, D.N., Caular, T., 2013. CSF control samples. Furthermore, we thank Carola Skärvinge and Anna Arvidsson at Stockholm Spine Center for excellent logistic assistance and for organizing the database and Azar Baharpoor, Department of Physiology and Pharmacology, Karolinska Institutet for support and laboratory assistance. We thank Stockholm Spine Center for providing research facilities for the study.

Daghnia, S., Tricco, A.C., Haldeman, S., 2010. Synthesis of recommendations for the assessment and management of low back pain from recent clinical practice guidelines. Spine J. 10, 514–529.

Devlin, N.J., Brooks, R., 2017. EQ-5D and the euroqol group: past, present and future. Appl. Health Econ. Health Policy 15, 127–137.

Doyle, H.H., Murphy, A.Z., 2017. Sex differences in innate immunity and its impact on opioid pharmacology. J. Neurosci. Res. 95, 487–499.

Elrich, L.C., Hu, S., Sheng, W.S., Sutton, R.L., Rockwood, G.L., Peterson, P.K., Chao, C.C., 1998. Cytokine regulation of human microglial cell IL-8 production. J. Immunol. 160, 1944–1948.

Ellis, A., Bennett, D.L., 2013. Neuroinflammation and the generation of neuropathic pain. Br. J. Anaesth. 111, 26–37.
Eriksson, A., Mannerkorpi, K. 2007. Assessment of fatigue in patients with fibromyalgia and chronic widespread pain. Reliability and validity of the Swedish version of the MFI-20. Disabil. Rehabil. 29, 1665–1670.
Fairbank, J.C., Cooper, J., Davies, J.B. 1980. The Oswestry low back pain questionnaire. Physiotherapy 66, 193–197.
Feng, C., Yang, M., Lan, M., Liu, C., Zhang, Y., Huang, B., Liu, H., Zhou, Y. 2017. ROS: crucial intermediators in the pathogenesis of intervertebral disc degeneration. Osteoarthr. Cartil. 25, 1251–1259.
Franco, R., Pacheco, R., Llanes, C., Ahern, G.P., O’Connell, P.J. 2007. The emergence of neurotransmitters as immune modulators. Trends Immunol. 28, 400–407.
Freemont, A., Watkins, A., Le Maître, C., Baird, P., Zejzorska, M., Knight, M.T., Ross, E.R., O’Brien, J.P., Hoyaland, J.D., 2002. Nerve growth factor expression and innervation of the human intervertebral disc. Arch. Pathol. 157, 286–292.
Freyhagen, R., Rolke, R., Baron, R., Töle, T.R., Rutjes, A.K., Schu, S., Treede, R.D., 2008. Pseudoradiculat and radicular low back pain—A disease continuum rather than different entities? Answers from quantitative sensory testing. Pain 135, 65–74.
Frieden, C., Thoer, U., Glessmark, B., Kosek, E., Nordmark, B., Lundberg, L.E., O’pava, C.H. 2013. Higher pain sensitivity and lower muscle strength in postmenopausal women with early rheumatoid arthritis compared with age-matched healthy women—a pilot study. Disabil. Rehabil. 35, 1350–1356.
Garrick, J., Campanella, M. 2015. TSPo is a REDD3 regulator of cell mitophagy. Biochim. Biophys. Soc. Trans. 43, 543–552.
Gent, Y.Y., Abrahm, N., Voskay, A.L., Hoejte, N., van Kuijk, C., Brittissem, K., Turkstra, F., Boers, M., Hoekstra, G.S., van der Laken, C.J., 2014. Detection of subclinical syphilitis in patients with macular degeneration and posterior atrophy in patients with rheumatoid arthritis without clinical arthritis. J. Rheumatol. 41, 2145–2152.
Grace, P., Hutchison, M., Maier, S., Watkins, L. 2014. Pathological pain and the neuroimmune interface. Nat. Rev. Immunol. 14, 217–231.
Greven, T., Le,a_s, T., Langford, C., Nielsen, L., Kid, K.L., 2012. Normalization of widespread hypertheresia and facilitated spatial summation of deep-tissue pain in knee osteoarthritis patients after knee replacement. Arthritis Rheum. 64, 2907–2916.
Greter, M., Heppner, F., Lemm, M.P., Ödemar, B.M., Goebels, N., Lauf, T., Noelle, R.J., Becker, B. 2005. DCdritic cells permit immune invasion of the CNS in an in vitro model of multiple sclerosis. Nat. Med. 11, 328–334.
Gutierrez, E.G., Banks, W.A., Canton, A.J., 1994. Blood-bone interleukin-1 receptor antagonist crosses the synovial barrier. J. Neuroimmunol. 55, 153–160.
Hamilton, M.M., Lapointe, G.R., Feucht, P.H., Hilt, S., Gallegos, C.A., Gordon, C.A., Purmessur, D., Freemont, A.J., Hoyland, J.A., 2008. Expression and regulation of cytokines in the nondegenerate and degenerate human intervertebral disc. Arthritis Res. Ther. 10, R213.
Hayashida, K., Nanki, T., Girschick, H., Vayev, S., Ochi, T., Lipsky, P.E., 2001. Synovial stromal cells from rheumatoid arthritis patients attract monocytes by producing MCP-1 and IL-8. Arthritis Res. Ther. 3, 118–126.
Ji, R.-R., Berta, T., Niedergaard, M. 2013. Glia and pain: is chronic pain a gliopathy? Pain 514, 510–528.
Ji, R.-R., Xu, Z.-Z., Gao, Y.-J., 2014. Emerging targets in neuroinflammation-driven chronic pain. Nat. Rev. Drug Discov. 13, 533–548.
Karshikoff, B., Lekander, M., Soop, A., Lindstedt, F., Ingvar, M., Kosek, E., 2015. Modality and sex differences in pain sensitivity during human endotoxemia. Brain Behav. Immun. 46, 35–43.
Koets, H.M., Adler, M., Schipper, W., van der Linden, P.J., Iatridis, J.C., 2018. Accumulation and localization of macrophage phenotypes with human intervertebral disc degeneration. Spine J. 18, 343–356.
Kong, S., O’connor, D., Wenzel, R.H., Velmans, L., Mak, K., 2013. The psychometric properties of the Swedish multidimensional fatigue inventory MFI-20 in four different populations. Acta Oncol. 46, 97–104.
Koizumi, H., Ito, H., Tsuchida, E., Ishiguro, S., Igarashi, S., Yamada, S., Yabe, K., 2016. Inflammatory serum protein profiling of patients with lumbar radicular pain one year after disc herniation. Int. J. Inflam. 2016, 3874964.
Kosek, E., Svensson, C.I., 2012. Peripheral inflammatory disease associated with centenarily activated IL-1 system in humans and mice. Proc. Natl. Acad. Sci. U.S.A. 109, 12728–12733.
Koizumi, H., Ito, H., Tsuchida, E., Ishiguro, S., Igarashi, S., Yamada, S., Yabe, K., 2016. Inflammatory serum protein profiling of patients with lumbar radicular pain one year after disc herniation. Int. J. Inflam. 2016, 3874964.
Ther. 16, 470.
Schistad, E.I., Espeland, A., Pedersen, I.M., Sandvik, L., Gjerstad, J., Ree, C., 2014. Association between baseline IL-6 and 1-year recovery in lumbar radicular pain. Eur. J. Pain 18, 1394–1401.
Schoenenfeld, A.J., Weiner, B.K., 2010. Treatment of lumbar disc herniation: evidence-based practice. Int. J. Gen. Med. 3, 209–214.
Shamji, M.F., Setton, L.A., Jarvis, W., So, S., Chen, J., Jing, L., Bullock, R., Isaacs, R.E., Brown, C., Richardson, W.J., 2010. Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. Arthritis Rheum. 62, 1974–1982.
Verma, S., Nakaoka, R., Dohgu, S., Banks, W.A., 2006. Release of cytokines by brain endothelial cells: a polarized response to lipopolysaccharide. Brain Behav. Immun. 20, 449–455.
Verma, V., Sheikh, Z., Ahmed, A.S., 2015. Nociception and role of immune system in pain. Acta Neurol. Belg. 115, 213–220.
Walsh, D.A., Mapp, P.I., Kelly, S., 2015. Calcitonin gene-related peptide in the joint: contributions to pain and inflammation. Br. J. Clin. Pharmacol. 80, 965–978.
Wang, K., Bao, J.P., Yang, S., Hong, X., Liu, L., Xie, X.H., Wu, X.T., 2016. A cohort study comparing the serum levels of pro- or anti-inflammatory cytokines in patients with lumbar radicular pain and healthy subjects. Eur. Spine J. 25, 1428–1434.
Wang, J., Markova, D., Anderson, D.G., Zheng, Z., Shapiro, I.M., Rishud, M.V., 2011. TNF-α and IL-1β promote a disintegrin-like and metalloprotease with thrombospondin type I motif-5-mediated aggrecan degradation through syndecan-4 in intervertebral disc. J. Biol. Chem. 286, 39738–39749.

Weber, K.T., Olivier Alipui, D., Sison, C.P., Bloom, O., Quraishi, S., Overby, M.C., Levine, M., Chahine, N.O., 2016. Serum levels of the proinflammatory cytokine interleukin-6 vary based on diagnoses in individuals with lumbar intervertebral disc diseases. Arthritis Res. Ther. 18, 3.
Whynes, D.K. for the TOMBOLA Group, 2008. Correspondence between EQ-5D health state classifications and EQ VAS scores. Health Quality Outcomes 6, 94.
Wu, F., Liu, L., Zhou, H., 2017. Endothelial cell activation in central nervous system inflammation. J. Leukoc. Biol. 101, 1119–1132.
Xanthis, D.N., Sandkühler, J., 2014. Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. Nat. Neurosci. 15, 43–53.
Yao, Y., Tsirka, S.E., 2014. Monocyte chemoattractant protein-1 and blood-brain barrier. Cell Mol. Life Sci. 71, 683–697.
Yoshida, M., Nakamura, T., Sei, A., Kikuchi, T., Takagi, K., Matsukawa, A., 2005. Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model. Spine (Phila Pa 1976) 30, 55–61.
Zhang, Y., Chee, A., Shi, P., Adams, S.L., Markova, D.Z., Anderson, D.G., Smith, H.E., Deng, Y., Plastaras, C.T., An, H.S., 2016. Intervertebral disc cells produce interleukins found in patients with back pain. Am. J. Phys. Med. Rehabil. 95, 407–415.
Zhang, Y.G., Guo, T.M., Guo, X., Wu, X.X., 2009. Clinical diagnosis for discogenic low back pain. Int. J. Biol. Sci. 5, 647–658.
Zheng, J., Shi, X.Q., Echeverry, S., Mogil, J.S., DeKoninck, Y., Rivest, S., 2007. Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. J. Neurosci. 27, 12396–12406.