

Elevated inflammatory proteins in cerebrospinal fluid from patients with painful knee osteoarthritis are associated with reduced symptom severity

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ABSTRACT

Neuroinflammation and periphery-to-CNS neuroimmune cross-talk in patients with painful knee osteoarthritis (OA) are poorly understood. We utilized proximity extension assay to measure the level of 91 inflammatory proteins in CSF and serum from OA patients and controls. The patients had elevated levels of 48 proteins in CSF indicating neuroinflammation. Ten proteins were correlated between CSF and serum and potentially involved in periphery-to-CNS neuroimmune cross-talk. Seven CSF proteins, all with previously reported neuroprotective effects, were associated with lower pain intensity and milder knee-related symptoms. Our findings indicate that neuroinflammation in OA could be protective and associated with less severe symptoms.

1. Introduction

Osteoarthritis (OA), the most prevalent form of arthritis, is characterized by pain, inflammation, destruction of joint tissues, and functional impairment (Martel-Pelletier et al., 2016). Pain is the primary symptom of OA and the main reason why OA patients decide to undergo a total knee replacement (TKR) (Eitner et al., 2017; Fu et al., 2018). Although new potentially promising pharmacological treatments may be underway (Miller et al., 2017), at present surgery is the only effective treatment for patients suffering from severe OA pain. Pain mechanisms in OA are complex, including peripheral (Schaible and Grubb, 1993; Hawker et al., 2008) as well as central sensitization (Kosek and Ordeberg, 2000a; Kosek and Ordeberg, 2000b; Arendt-Nielsen et al., 2010) and inflammation (Miller et al., 2014). A better understanding of pain mechanisms is therefore crucial to provide patients with an adequate pain relief (Malfait and Schnitzer, 2013).

The important role of the neuroimmune interface in chronic pain is increasingly recognized (Grace et al., 2014). The contribution of cytokines to joint pain has been documented both in animal models of OA and in clinical studies with OA patients (Hill et al., 2007; Miller et al., 2014). For example, when injected into the knees of healthy rats, the

pro-inflammatory cytokines tumor necrosis factor alpha (TNF- α) and interleukin (IL)-6 sensitized the knee innervating nociceptive fibers to mechanical stimuli (Brenn et al., 2007; Richter et al., 2010) and correspondingly, the synovial fluid (SF) concentrations of these cytokines were associated with more intense pain in knee OA patients (Orita et al., 2011; Kosek et al., 2018). The pro-inflammatory substances, such as cytokines and chemokines, are not only released locally in the joints, they are also released in dorsal root ganglion (DRG) and dorsal horn of the spinal cord by glial cells and nociceptive neurons (Miller et al., 2014). In accordance with this, increased levels of IL-6 and IL-8 in the SF (Monibi et al., 2016; Siqueira et al., 2017; Kosek et al., 2018; Nees et al., 2019) as well as higher concentrations of IL-8, IL-1 β and monocyte chemoattractant protein 1 (MCP-1) in the cerebrospinal fluid (CSF) (Lundborg et al., 2010; Kosek et al., 2018) have been documented in knee OA patients. Furthermore, the concentration of MCP-1, a chemokine increasing blood brain barrier (BBB) permeability (Stamatovic et al., 2005; Yao and Tsirka, 2014), was positively correlated across CSF, serum and SF in female OA patients (Kosek et al., 2018) as well as across CSF and serum in patients suffering from disc degenerative disease (Palada et al., 2019), indicating the presence of blood-borne periphery-to-central nervous system (CNS) neuroimmune cross-talk.

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Table 1
Characteristics of study participants.

| | OA (N = 40) | HC (N = 40) | CSF controls (N = 40) | OA vs HC Diff | OA vs CSF controls Diff |
|--------------------------|---------------------|---------------------|-----------------------|----------------------|-------------------------|
| Age (years) | 64.5 (49–73) | 64.8 (49–73) | 47.35 (26–73) | NS | P < 0.0001 |
| BMI (kg/m ²) | 27.9 (21.6–36.3) | 25 (19.6–31.6) | NA | P = 0.0004 | NA |
| VAS global (mm) | 41.3 (0–100) | 2.15 (0–24) | NA | P < 0.0001 | NA |
| VAS knee (mm) | 17.7 (0–75) | 0 (0–0) | NA | P < 0.0001 | NA |
| KOOS | 36.2 (7.7–57.8) | 97.8 (81.25–100) | NA | P < 0.0001 | NA |
| PPT knee (kPa) | 347.2 (131–589) | NA | NA | NA | NA |
| PPT average (kPa) | 451.2 (144.6–797.2) | 410.9 (223.6–806.9) | NA | <i>P = 0.35</i> | NA |
| CPM score | 0.29 (–0.39–1.32) | 0.34 (–0.07–1.17) | NA | <i>P = 0.89</i> | NA |

The values are presented as means, with the indicated minimum and maximum. Mann-Whitney *U* test was used for the between group comparisons. OA = Patients with knee osteoarthritis; HC = Healthy controls; CSF controls = Patients with non-inflammatory neurological symptoms. BMI = Body mass index; VAS global = Visual analogue scale for global pain (0–100; 0 = no pain; 100 = the worst imaginable pain); VAS knee = Visual analogue scale for pain in the affected knee (0–100; 0 = no pain; 100 = the worst imaginable pain); KOOS = Knee injury and Osteoarthritis Outcome Score (0–100; 0 = the worst symptoms; 100 = no symptoms); PPT knee = Pressure pain threshold at the affected knee; PPT average = Average pressure pain threshold from bilateral assessment of trapezius and gluteal muscles; CPM score = Conditioned pain modulation score; increase in PPTs at the end of the conditioning stimulus compared to baseline. Diff = Group difference; NS = not significant (*p* > 0.05); NA = not available. Bold indicates significant differences.

Traditionally, the neuroimmune interface has been implicated in pain generating mechanisms such as enhanced excitability of nociceptive neurons and opioid induced hyperalgesia, however, the neuroprotective aspects of neuroimmune signaling are increasingly recognized (Grace et al., 2014). Recently, we reported that IL-6 and IL-8 levels in CSF were inversely associated with symptom severity in knee OA patients, indicating central analgesic and/or neuroprotective effects of these proteins (Kosek et al., 2018). However, the previous studies only examined a very limited number of substances. In the present study, proximity extension assay (PEA), which allows for the simultaneous analysis of 91 human inflammatory proteins, predominantly cytokines and chemokines (Lundberg et al., 2011; Assarsson et al., 2014) was used to analyze CSF and serum from OA patients and controls. We hypothesized that: 1) OA patients have elevated levels of inflammatory proteins in CSF and serum, 2) the levels of certain proteins correlate across CSF and serum, indicating potential involvement in blood-borne neuroimmune crosstalk and 3) specific proteins in CSF will be associated with lower pain and symptom severity, reduced pain sensitivity and/or better function of descending pain inhibitory mechanisms, indicating central analgesic effects.

2. Materials and methods

2.1. Subjects

Forty patients with knee OA (17 women and 23 men, average age 64.5 years, range 49–73 years) and forty healthy controls (HC; 20 women and 20 men; average age 64.3 years, range 49–73 years) participated. All patients were recruited consecutively from the waiting list for TKR at Ortho Center, Upplands Väsby, Sweden while controls were recruited by advertisement in the local newspapers. The patient inclusion criteria were 25–75 years of age, radiologically verified knee OA and the presence of knee pain as the dominant pain symptom and a motivation for surgery. The patients were excluded if they suffered from chronic pain due to causes other than knee OA (e.g., fibromyalgia, degenerative disc disease, disc herniation, inflammatory rheumatic disease or neurologic disease) or in case of previous knee surgery at the knee planned for total knee replacement. The exclusion criteria for HCs were the same as for the patients, but in addition HCs were excluded if they had a diagnosis of OA or an average weakly pain rating of > 20 mm on a 100 mm visual analogue scale (VAS). Information regarding medication was collected from all patients. Eight patients were taking analgesics (3 codeine, 2 tramadol, 2 buprenorphin plaster, 1 Ketobemidone), 14 were taking acetaminophen and 18 had previously been taking nonsteroidal anti-inflammatory drugs (NSAIDs) at demand, however these had been stopped 14 days before the surgical procedure. All patients received 2 g acetaminophen (paracetamol) and 10 mg

oxycodone orally as premedication before surgery. CSF and serum samples were taken from patients and serum samples were obtained from HCs.

In addition, 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 (NINS) 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 Magnetic Resonance Imaging (MRI) 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.

Data from the same cohorts (OA patients, HC and NINS) regarding a limited number of cytokines/chemokines (IL-6, IL-8 and MCP1) assessed with a different method (Meso Scale Discovery immunoassay) have previously been published (Kosek et al., 2018).

The procedures were approved by the local ethical committee (2011/2036-31-1) and all participants have signed the written consent.

2.2. Procedure

The patients filled the questionnaires and pressure pain sensitivity as well as conditioned pain modulation was assessed, usually within a week from the surgery. On the day of surgery, prior to the surgical procedure, venous blood samples were collected from the antecubital vein and lumbar puncture was performed to collect CSF before spinal analgesia.

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. For ethical reasons we had to rely on separate controls for CSF.

2.3. Questionnaires

Global pain intensity at the day of examination (VAS global) and pain in the affected knee (VAS knee) were all scored using 100 mm VAS with 0 indicating “no pain” and 100 indicating “the worst imaginable pain”. The severity of patient-reported symptoms was assessed by Knee injury and Osteoarthritis Outcome Score (KOOS) which consists of 5 subscales: a) pain, b) other symptoms, c) activity in daily living, d) function in sport and recreation, and e) knee related quality of life (Roos et al., 1998; Roos and Toksvig-Larsen, 2003). Each KOOS

Table 2
Normalized protein expression (NPX) values for CSF and serum proteins with significant group differences.

| Protein name | Gene | CSF | | | | Gene | Serum | | | |
|---|-----------------|-------------|-----------------------|-----------------------------|-----------------------------|-----------------|-------------|-------------|-------------------|-------------------|
| | | OA (n = 40) | CSF controls (n = 37) | OA vs CSF controls p values | OA vs CSF controls q values | | OA (n = 39) | HC (n = 37) | OA vs HC p values | OA vs HC q values |
| Eukaryotic translation initiation factor 4E-binding protein 1 | 4E-BP1 | 1.895 | 1.324 | 0.0001 | 0.0006 | 4E-BP1 | 6.915 | 6.759 | 0.65 | 0.85 |
| Adenosine deaminase | ADA | 3.545 | 2.959 | 0.0001 | 0.0006 | ADA | 3.732 | 3.678 | 0.46 | 0.68 |
| Beta-nerve growth factor | Beta-NGF | 1.750 | 1.451 | 0.0003 | 0.0013 | Beta-NGF | 1.727 | 1.837 | 0.64 | 0.85 |
| C-C motif chemokine 3 | CCL3 | 2.789 | 2.479 | 0.0183 | 0.0282 | CCL3 | 5.378 | 5.137 | 0.03 | 0.13 |
| C-C motif chemokine 4 | CCL4 | 3.596 | 3.324 | 0.0509 | 0.0631 | CCL4 | 7.450 | 7.201 | 0.0498 | 0.19 |
| C-C motif chemokine 11 | CCL11 | 1.639 | 1.513 | 0.0446 | 0.0598 | CCL11 | 8.177 | 8.275 | 0.2 | 0.52 |
| C-C motif chemokine 19 | CCL19 | 8.371 | 7.451 | 0.0001 | 0.0006 | CCL19 | 9.021 | 8.803 | 0.11 | 0.34 |
| C-C motif chemokine 23 | CCL23 | 2.306 | 1.908 | 0.0001 | 0.0006 | CCL23 | 10.000 | 9.665 | 0.0009 | 0.009 |
| C-C motif chemokine 25 | CCL25 | 0.953 | 0.742 | 0.0041 | 0.009 | CCL25 | 6.546 | 6.435 | 0.33 | 0.62 |
| C-C motif chemokine 28 | CCL28 | 0.340 | 0.195 | 0.0157 | 0.0256 | CCL28 | 1.316 | 1.296 | 0.54 | 0.78 |
| T-cell surface glycoprotein CD5 | CD5 | 0.865 | 0.647 | 0.0198 | 0.0297 | CD5 | 4.659 | 4.685 | 0.83 | 0.89 |
| CD40L receptor | CD40 | 7.581 | 7.317 | 0.0098 | 0.0169 | CD40 | 9.936 | 9.728 | 0.0081 | 0.0822 |
| Natural killer cell receptor 2B4 | CD244 | 0.791 | 0.451 | 0.0014 | 0.0036 | CD244 | 5.573 | 5.626 | 0.7 | 0.85 |
| CUB domain-containing protein 1 | CDCP1 | 3.603 | 3.477 | 0.61 | 0.61 | CDCP1 | 3.547 | 3.184 | 0.0094 | 0.0822 |
| Macrophage colony-stimulating factor 1 | CSF-1 | 7.149 | 6.874 | 0.0029 | 0.0069 | CSF-1 | 7.961 | 7.888 | 0.15 | 0.43 |
| Cystatin D | CST5 | 5.186 | 5.018 | 0.0001 | 0.0006 | CST5 | 5.854 | 6.072 | 0.06 | 0.2 |
| Fractalkine | CX3CL1 | 2.433 | 2.072 | 0.0005 | 0.002 | CX3CL1 | 5.576 | 5.480 | 0.43 | 0.66 |
| C-X-C motif chemokine 1 | CXCL1 | 4.462 | 4.090 | 0.0150 | 0.0251 | CXCL1 | 8.449 | 8.555 | 0.26 | 0.58 |
| C-X-C motif chemokine 6 | CXCL6 | 3.264 | 2.755 | 0.0020 | 0.005 | CXCL6 | 8.376 | 8.419 | 0.93 | 0.96 |
| C-X-C motif chemokine 9 | CXCL9 | 3.829 | 2.852 | 0.0001 | 0.0006 | CXCL9 | 7.728 | 7.793 | 0.84 | 0.89 |
| C-X-C motif chemokine 10 | CXCL10 | 7.910 | 6.999 | 0.0002 | 0.0011 | CXCL10 | 8.616 | 8.334 | 0.06 | 0.2 |
| C-X-C motif chemokine 11 | CXCL11 | 2.901 | 2.341 | 0.0013 | 0.0035 | CXCL11 | 8.004 | 7.965 | 0.66 | 0.85 |
| Delta and Notch-like epidermal growth factor related receptor | DNER | 9.739 | 9.586 | 0.0003 | 0.0013 | DNER | 8.093 | 8.148 | 0.37 | 0.64 |
| Fibroblast growth factor 5 | FGF-5 | 3.186 | 3.009 | 0.0600 | 0.0728 | FGF-5 | 1.160 | 1.170 | 0.83 | 0.89 |
| Fibroblast growth factor 19 | FGF-19 | 4.140 | 3.855 | 0.0497 | 0.063 | FGF-19 | 7.825 | 8.598 | 0.0001 | 0.002 |
| Fibroblast growth factor 21 | FGF-21 | 0.963 | 0.797 | 0.0003 | 0.0013 | FGF-21 | 5.796 | 4.857 | 0.0002 | 0.003 |
| Fibroblast growth factor 23 | FGF-23 | 0.229 | 0.305 | 0.0001 | 0.0006 | FGF-23 | 1.147 | 1.003 | 0.33 | 0.62 |
| Fms-related tyrosine kinase 3 ligand | Flt3L | 8.161 | 7.783 | 0.0010 | 0.0032 | Flt3L | 8.878 | 9.087 | 0.013 | 0.0822 |
| Hepatocyte growth factor | HGF | 7.801 | 7.432 | 0.0070 | 0.0128 | HGF | 8.820 | 8.569 | 0.0065 | 0.0822 |
| Interleukin-7 | IL7 | 1.456 | 1.292 | 0.0169 | 0.0268 | IL7 | 4.936 | 4.939 | 0.98 | 0.98 |
| Interleukin-8 | IL8 | 7.970 | 7.635 | 0.0072 | 0.0128 | IL8 | 6.559 | 6.833 | 0.0148 | 0.11 |
| Interleukin-10 receptor subunit beta | IL-10RB | 3.095 | 2.596 | 0.0006 | 0.0023 | IL-10RB | 6.517 | 6.427 | 0.80 | 0.89 |
| Interleukin-18 | IL18 | 1.414 | 1.203 | 0.0209 | 0.0298 | IL18 | 7.708 | 7.718 | 0.82 | 0.89 |
| Interleukin-18 receptor 1 | IL-18R1 | 1.896 | 1.601 | 0.0497 | 0.063 | IL-18R1 | 7.119 | 6.704 | 0.0001 | 0.002 |
| Leukemia inhibitory factor receptor | LIF-R | 2.828 | 2.536 | 0.0068 | 0.0128 | LIF-R | 2.770 | 2.738 | 0.64 | 0.85 |
| Monocyte chemoattractant protein 2 | MCP-2 | 3.920 | 3.493 | 0.0204 | 0.0298 | MCP-2 | 8.807 | 8.671 | 0.38 | 0.64 |
| Matrix metalloproteinase-10 | MMP-10 | 1.206 | 0.868 | 0.0012 | 0.0035 | MMP-10 | 5.861 | 5.937 | 0.68 | 0.85 |
| Osteoprotegerin | OPG | 10.040 | 9.660 | 0.0062 | 0.0122 | OPG | 10.490 | 10.390 | 0.26 | 0.58 |
| Programmed cell death 1 ligand 1 | PD-L1 | 3.692 | 3.207 | 0.0008 | 0.0027 | PD-L1 | 3.727 | 3.611 | 0.041 | 0.17 |
| Stem cell factor | SCF | 4.946 | 4.540 | 0.0013 | 0.0035 | SCF | 9.709 | 9.795 | 0.24 | 0.58 |
| SIR2-like protein 2 | SIRT2 | 0.830 | 0.454 | 0.0008 | 0.0027 | SIRT2 | 1.350 | 1.290 | 0.94 | 0.96 |
| STAM-binding protein | STAMPB | 0.718 | 0.563 | 0.0001 | 0.0006 | STAMPB | 3.569 | 3.470 | 0.31 | 0.62 |
| Tumor necrosis factor beta | TNFB | 0.631 | 0.421 | 0.0215 | 0.0299 | TNFB | 3.724 | 3.799 | 0.29 | 0.62 |
| Tumor necrosis factor ligand superfamily member 9 | TNFRSF9 | 2.025 | 1.828 | 0.0451 | 0.0598 | TNFRSF9 | 6.138 | 6.020 | 0.2 | 0.52 |
| Tumor necrosis factor ligand superfamily member 14 | TNFSF14 | 1.321 | 0.839 | 0.0001 | 0.0006 | TNFSF14 | 5.910 | 5.792 | 0.41 | 0.65 |
| TNF-related apoptosis-inducing ligand | TRAIL | 1.911 | 1.761 | 0.0786 | 0.0933 | TRAIL | 8.505 | 8.433 | 0.71 | 0.85 |
| Tumor necrosis factor ligand superfamily member 12 | TWEAK | 10.220 | 9.859 | 0.0040 | 0.009 | TWEAK | 9.567 | 9.539 | 0.39 | 0.64 |
| Urokinase-type plasminogen activator | uPA | 7.299 | 6.971 | 0.0058 | 0.0118 | uPA | 10.000 | 10.030 | 0.35 | 0.64 |
| Vascular endothelial growth factor A | VEGFA | 8.951 | 8.599 | 0.0055 | 0.0116 | VEGFA | 10.390 | 10.170 | 0.0081 | 0.0822 |

Data are presented as means of the NPX values. Mann-Whitney U test was used for the between group comparisons and the p values as well as the FDR corrected p values (q values) are presented for all the proteins significant at the 10% FDR level. CSF = cerebrospinal fluid, OA = knee osteoarthritis patients, HC = healthy controls, NS = Not significant. Q values in bold indicate significant differences.

subscale contains questions scored from 0 to 4 summarized into continuous scores ranging from 0 (worst) to 100 (best) and the average score of all 5 KOOS subscales was calculated and used for the analysis.

2.4. Sensory testing protocol

Sensitivity to pressure pain was assessed by a pressure algometer

(Somedic Sales AB, Hörby, Sweden) with a flat circular tip area of 1 cm² and aiming for a constant pressure increase of approximately 50 kPa/s using a visual feedback (Kosek et al., 1993). To assess pressure pain thresholds (PPTs) subjects were asked to press a button as soon as the pressure became painful. The PPTs were assessed at the medial epicondyle of femur, close to the knee joint space (PPTknee). In order to obtain a measure of the general pain sensitivity, PPTs were also

Table 3
Correlation of protein levels between CSF and serum.

| Protein name | Gene | Rho | p-value | q-value |
|--------------------------------------|---------|------|----------|---------|
| C-C motif chemokine 25 | CCL25 | 0.65 | 6.62E-06 | 0.00038 |
| C-X-C motif chemokine 9 | CXCL9 | 0.62 | 4.09E-05 | 0.00089 |
| Interleukin-12 subunit beta | IL-12B | 0.61 | 4.68E-05 | 0.00089 |
| Fibroblast growth factor 21 | FGF-21 | 0.58 | 0.00011 | 0.0015 |
| Adenosine deaminase | ADA | 0.46 | 0.0037 | 0.036 |
| Interleukin-18 receptor 1 | IL-18R1 | 0.46 | 0.0038 | 0.036 |
| Interleukin-10 receptor subunit beta | IL-10RB | 0.44 | 0.005 | 0.036 |
| C-C motif chemokine 4 | CCL4 | 0.44 | 0.0051 | 0.036 |
| Urokinase-type plasminogen activator | uPA | 0.42 | 0.0084 | 0.053 |
| C-C motif chemokine 11 | CCL11 | 0.38 | 0.017 | 0.098 |

Spearman's rank correlation coefficient (Rho). The FDR adjusted p-values (or q-values) < 0.1 are considered significant.

assessed once/site, bilaterally, at the trapezius muscle (mid-point of the upper border) and gluteal muscle (upper outer quadrants of buttocks in anterior fold of muscle) and the average of these assessments was calculated for each participant (PPTaverage).

Conditioned pain modulation (CPM) was assessed using the cold pressor test. The participants placed their left forearm and hand in an ice water tub of 0–1 °C (conditioning stimuli) and PPTs at the right thigh were determined before (baseline) and then every 15 s for 5 min or until the participant withdrew the forearm from the tub due to intense pain. The relative increase in PPTs at the end of the conditioning stimulus (last PPT) compared to baseline (first assessment) was calculated as a measure of CPM (CPMscore = PPTend/PPTbaseline) (Kosek and Lundberg, 2013).

2.5. Sample collection and storage

Intravenous blood was collected in 2 × 8.5 ml BD Vacutainer® SST II plastic tube, incubated at room temperature for 30–40 min and centrifuged at 2500 rpm for 10 min at room temperature. The serum was collected, aliquoted and stored at –80 °C until further analysis. The CSF was collected without any additive, immediately centrifuged at 2500 rpm at room temperature and the supernatant was aliquoted and frozen at –80 °C for future analysis.

2.6. Proximity extension assay

CSF and serum samples were analyzed by multiplexed PEA immunoassay using Proseek Multiplex inflammation panel (Olink Proteomics, Uppsala, Sweden) which allows the simultaneous analysis of 91 inflammation-related proteins across 96 samples (Lundberg et al., 2011; Assarsson et al., 2014). Briefly, 1 µl of samples (CSF or serum) or negative controls were prepared in randomized 96 well plates, mixed with 3 µl of solution containing a set of 91 DNA-oligonucleotide-conjugated antibodies and incubated overnight at 8 °C. After incubation, 96 µl extension solution containing PEA enzyme and the reagents for polymerase chain reaction (PCR) were added to the mixture, incubated for 5 min at room temperature and transferred to microfluidic real-time quantitative PCR instrument BioMark HD System (Fluidigm, San Francisco, USA) for amplification and detection according to the instructions from Proseek Multiplex. The data was expressed on log2 scale as normalized protein expression (NPX) values by normalizing quantification cycle (Cq) values against the spiked-in controls, inter-plate control and a correction factor. The NPX values show a positive correlation to the protein concentrations allowing the relative protein quantification between the samples. Limit of detection (LOD) was defined as 3 standard deviations above the background.

2.7. Statistical analysis

Proteins that were detected (above LOD) in at least 50% of the

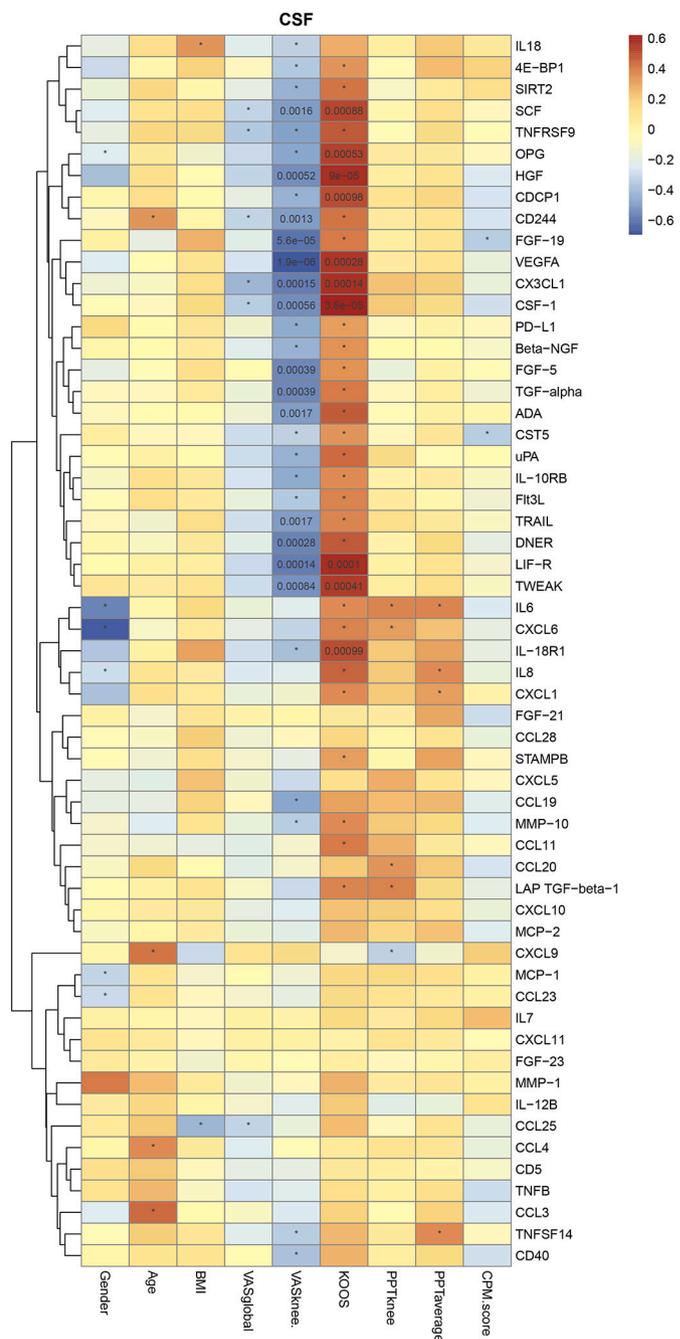


Fig. 1. Association of CSF and serum proteins with clinical parameters. The associations according to Spearman's rank correlation test between CSF (a) and serum (b) and (from left) gender, age, BMI, VAS ratings of global pain (VASglobal), VAS ratings of knee pain (VASknee), ratings of knee related symptoms (KOOS), pressure pain thresholds at the affected knee (PPTknee), average of 4 PPT assessments shoulders and buttocks, bilaterally (PPTaverage) and the amount of conditioning pain modulation (CPMscore). The p values are listed for correlations that are statistically significant at the 0.1 level after Bonferroni correction. Associations with p < 0.05 before correction are marked with *. Red illustrates positive correlations and blue negative. More severe pain/symptoms are associated with higher VAS ratings but lower KOOS values. Low PPT values = higher pain sensitivity and low CPM = less functional endogenous pain modulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

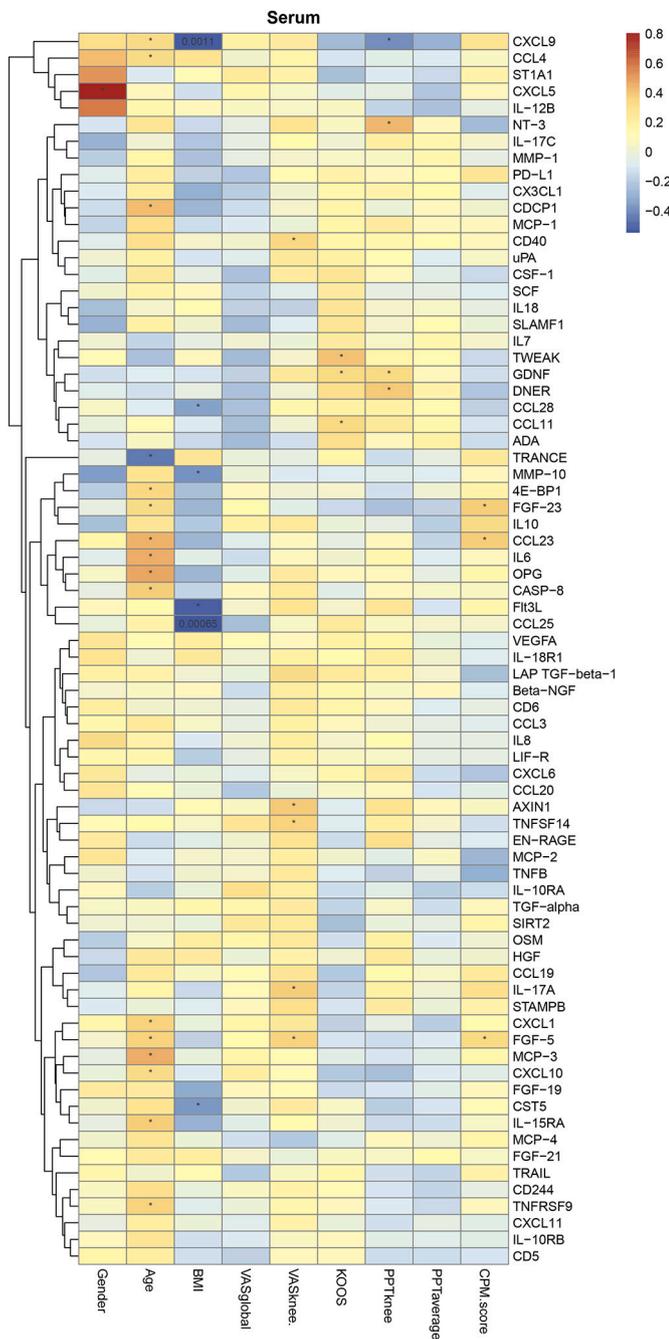


Fig. 1. (continued)

samples were included in the analysis. The principal component analysis (PCA) was performed based on all CSF and serum samples to get a data overview and to identify the potential plate effects. The CSF NPX values from OA patients were analyzed in comparison to CSF controls (NINS) and the serum values in comparison to healthy age- and sex matched controls (HC). Mann-Whitney *U* test was used for the between group comparisons. The correlation of the protein levels between CSF and serum was assessed by Spearman's rank correlation test. The analysis of group differences in protein levels and correlations between CSF and serum were corrected for multiple comparisons using False Discovery Rate (FDR) and were considered significant if the FDR adjusted *p*-values (or *q*-values) were below 0.1. The association between the protein level and age, body mass index (BMI) and the clinical parameters (VASglobal, VASKnee, KOOS, PPTaverage, PPTknee and CPMscore) was measured with Spearman's rank correlation test and

between protein level and gender with Mann-Whitney's *U* test. Significance levels $0.1/57 = 0.00175$ or $0.1/74 = 0.00135$ were applied for CSF and serum analyses, respectively, according to Bonferroni's method for multiple testing correction. Additionally, the association between protein levels and VASKnee ratings were assessed using linear regression adjusting for a subset of the covariates; age, gender, plate effect and BMI; that were moderately associated to the protein level $p < 0.05$, according to Spearman's correlation (age and BMI), Mann-Whitney's *U* test (gender) or Kruskal-Wallis test (plate effect). In the linear regression VASKnee values were discretized into 3 subgroups: 0 to 5 mm (no pain), 6 to 45 mm (mild pain) and 46 to 75 mm (moderate pain) (Jensen et al., 2003). The associations were considered significant if the FDR corrected ANOVA *p*-values (or *q* values) were below 0.1. The data were statistically analyzed using R software.

3. Results

3.1. Subject characteristics and clinical symptoms

Characteristics of OA patients, HC and CSF controls are listed in Table 1. Patients with knee OA had significantly higher global pain intensities (VASglobal) compared to HC ($p < 0.0001$) as well as higher knee pain intensities (VASKnee) ($p < 0.0001$). Furthermore, OA patients had pronounced knee related symptoms evidenced by reduced KOOS scores versus HC ($p < 0.0001$). No differences were observed between our cohort of OA patients and HC group regarding general pain sensitivity (PPT average) and conditioned pain modulation (CPM scores) Table 1. In the present study, CSF controls were on the average 17 years older than OA and HC groups and OA patients had higher BMI compared to HC ($p = 0.0004$). The duration of the current OA pain was < 1 year for 3 patients, 1–2 years for 6 patients and > 2 years for 31 patients.

3.2. Group differences in CSF and serum levels of inflammatory proteins

The 91 proteins measured in Proseek Multiplex Inflammation panel together with respective LOD values are listed in Supplementary Table 1. In total, 57 proteins in CSF and 74 serum proteins were detected above LOD in at least 50% of the samples and were included for the analysis (Supplementary Fig. 1). Majority of the samples have passed the quality control criteria except for one serum sample from OA patients, 3 serum samples from HCs and 3 CSF control samples. The PCA plots showed a clear difference in the protein profiles between the CSF and serum and there were no significant plate effects (Supplementary Fig. 2).

The proteins with significant group differences in CSF or serum levels are presented in Table 2. A total of 48 inflammatory proteins were elevated in CSF from OA patients compared to CSF controls, whereas no proteins with lower levels in CSF from OA patients were found. Compared to HC, OA patients had higher serum levels of 7 proteins and lower serum levels of two (Table 2; Supplementary Fig. 3).

3.3. Proteins with significant association between CSF and serum

Spearman's rank correlation test adjusted for multiple comparisons with FDR identified 10 inflammation associated proteins with significantly positive correlation between CSF and serum in OA patients (q -value < 0.1): adenosine deaminase (ADA), C–C motif chemokine 4 (CCL4), C–C motif chemokine 11 (CCL11), C–C motif chemokine 25 (CCL25), C–X–C motif chemokine 9 (CXCL9), fibroblast growth factor 21 (FGF-21), interleukin-10 receptor subunit beta (IL-10RB), interleukin-12 subunit beta (IL-12B), interleukin-18 receptor 1 (IL-18R1) and urikinase-type plasminogen activator (uPA) (Table 3; Supplementary Fig. 3). The strongest association was found for CCL25 ($r = 0.65$; $p = 6.62E-06$; $q = 0.00038$). Out of the 10 proteins, all except IL-12B,

Table 4

The associations between the CSF levels of the top proteins to pain ratings (VASknee) and knee-related symptoms (KOOS).

| VASknee | | | | | KOOS | | | | |
|-----------|----|--------|---------|---------|----------------|----|--------|---------|-------|
| Gene | Df | F | p | q | Gene | Df | F | p | q |
| VEGFA | 2 | 16,574 | 9.5e-06 | 0.00054 | CSF-1 | 1 | 15,995 | 0.00029 | 0.012 |
| DNER | 2 | 12,962 | 6.5e-05 | 0.0019 | CX3CL1 | 1 | 15,292 | 0.00043 | 0.012 |
| LIF-R | 2 | 9648 | 0.00048 | 0.0063 | TWEAK | 1 | 13,246 | 0.00083 | 0.012 |
| TWEAK | 2 | 9617 | 0.00049 | 0.0063 | OPG | 1 | 13,328 | 0.00092 | 0.012 |
| CX3CL1 | 2 | 9745 | 0.00055 | 0.0063 | HGF | 1 | 12,619 | 0.0011 | 0.012 |
| FGF-5 | 2 | 8700 | 0.00089 | 0.0084 | VEGFA | 1 | 11,676 | 0.0016 | 0.015 |
| TGF-alpha | 2 | 7906 | 0.0015 | 0.01 | LIF-R | 1 | 11,143 | 0.0019 | 0.015 |
| FGF-19 | 2 | 7862 | 0.0016 | 0.01 | CCL25 | 1 | 11,487 | 0.002 | 0.015 |
| CCL-19 | 2 | 8066 | 0.0016 | 0.01 | uPA | 1 | 10,875 | 0.0023 | 0.015 |
| PD-L1 | 2 | 7604 | 0.0019 | 0.011 | CCL11 | 1 | 9616 | 0.0039 | 0.021 |
| CSF-1 | 2 | 7447 | 0.0021 | 0.011 | IL-18R1 | 1 | 9375 | 0.0041 | 0.021 |
| ADA | 2 | 7293 | 0.0023 | 0.011 | SCF | 1 | 8869 | 0.0051 | 0.023 |
| SCF | 2 | 6578 | 0.0038 | 0.016 | CDCP1 | 1 | 8749 | 0.0054 | 0.023 |
| OPG | 2 | 6753 | 0.0039 | 0.016 | DNER | 1 | 8666 | 0.0056 | 0.023 |
| HGF | 2 | 5957 | 0.0061 | 0.023 | ADA | 1 | 8504 | 0.006 | 0.023 |
| CD244 | 2 | 5856 | 0.0067 | 0.024 | CST5 | 1 | 7771 | 0.0087 | 0.031 |
| Beta-NGF | 2 | 5582 | 0.008 | 0.027 | TNFRSF9 | 1 | 7206 | 0.011 | 0.034 |
| TRAIL | 2 | 5595 | 0.0086 | 0.027 | CXCL1 | 1 | 7123 | 0.011 | 0.034 |
| TNFRSF9 | 2 | 5290 | 0.01 | 0.03 | IL-8 | 1 | 7137 | 0.011 | 0.034 |
| CD40 | 2 | 5156 | 0.011 | 0.031 | IL-6 | 1 | 6814 | 0.014 | 0.036 |
| CDCP1 | 2 | 5115 | 0.011 | 0.031 | FGF-19 | 1 | 6568 | 0.015 | 0.036 |
| uPA | 2 | 5126 | 0.012 | 0.031 | LAP TGF-beta-1 | 1 | 6565 | 0.015 | 0.036 |
| 4E-BP1 | 2 | 4283 | 0.023 | 0.057 | Flt3L | 1 | 6480 | 0.015 | 0.036 |
| 1CCL3 | 2 | 3912 | 0.03 | 0.071 | CXCL6 | 1 | 6576 | 0.015 | 0.036 |
| SIRT2 | 2 | 3720 | 0.035 | 0.079 | TRAIL | 1 | 6258 | 0.017 | 0.04 |
| | | | | | CD244 | 1 | 5526 | 0.024 | 0.053 |
| | | | | | TGF-alpha | 1 | 4831 | 0.034 | 0.072 |
| | | | | | MCP-2 | 1 | 4188 | 0.048 | 0.097 |

ANOVA adjusted for age, gender, plate effect and BMI. The ANOVA Df, F, p value and FDR corrected p value (q value) is presented for all the proteins significant at the 10% FDR level. VAS knee = Visual analogue scale for pain in the affected knee (0–100; 0 = no pain; 100 = the worst imaginable pain); KOOS = Knee injury and Osteoarthritis Outcome Score (0–100; 0 = the worst symptoms; 100 = no symptoms).

had significantly higher levels in CSF from OA patients compared to CSF controls and FGF-21 and IL-18R1 levels were higher also in the serum from patients compared to HCs (Table 2). Regarding MCP1, we previously reported a weak, but significant correlation between CSF and serum in OA patients, which was driven by a positive correlation in women, but not men (Kosek et al., 2018). The OLINK analysis revealed no statistically significant correlations between CSF and serum MCP1 (all patients: $r = 0.25$, $p = 0.12$, men: $r = 0.09$, $p = 0.68$, women: $r = 0.459$, $p = 0.074$, uncorrected for multiple comparisons, readouts not fulfilling OLINK quality control removed).

3.4. Association of CSF and serum proteins with clinical parameters

We examined the association between the protein levels in CSF and serum with global pain intensity (VASglobal), knee pain intensity (VASknee), knee related symptom severity (KOOS), pressure pain sensitivity at the affected knee (PPTknee), general pressure pain sensitivity (PPTaverage), and efficacy of conditioned pain modulation (CPMscore). The associations according to Spearman's rank correlation test adjusted by Bonferroni correction for multiple testing are shown in Fig. 1. In general, several proteins in CSF showed a significant negative association with symptom severity, which was not observed for the proteins in serum.

In total, there were 14 proteins in CSF negatively correlated with VAS knee pain ratings and 10 proteins with significant positive association with KOOS scores (milder symptoms) (Fig. 1) and these associations were significant also after adjusting for covariates (age, gender, plate effect and/or BMI) (Table 4). All of these proteins, except for TGF-alpha and CDCP1, were elevated in the CSF of OA patients compared to CSF controls (Table 2). In addition, there was a significant association between the CSF and serum levels of ADA and IL-18RA in the OA patients.

Seven proteins in the CSF were significantly associated both with lower intensity of knee pain (negative correlation to VASknee) as well as with milder symptoms (positive correlation to KOOS): macrophage colony-stimulating factor 1 (CSF-1), fractalkine (CX3CL1), hepatocyte growth factor (HGF), leukemia inhibitory factor receptor (LIF-R), stem cell factor (SCF), fibroblast growth factor 21 (TWEAK) and vascular endothelial growth factor A (VEGFA) (Fig. 2; Supplementary Fig. 3). When corrected for covariates, we observed that CSF levels of these inflammatory proteins were lower in patients with moderate knee pain (VASknee = 45–74 mm) compared to patients without pain (VASknee = 0–4 mm) or with mild pain (VASknee = 5–44 mm) (Fig. 2). No proteins in CSF revealed a positive association to VASknee or VASglobal pain ratings or a negative correlation with KOOS and no significant correlations were found between the proteins in CSF and pressure pain sensitivity or endogenous pain modulation (CPM) (Fig. 1).

No significant associations were found between the protein levels in serum and clinical symptoms (Fig. 1). However, CXCL9 was significantly correlated to BMI and after adjusting for BMI and gender a significant positive association between CXCL9 and knee pain was detected (Df = 2, F = 14,49, $p = 0.000043$, $q = 0.0032$; VASknee).

4. Discussion

In accordance with our hypothesis, 48 inflammatory proteins were elevated in the CSF of OA patients, while no proteins had lower levels, which is in line with previous reports of neuroinflammation in OA (Lundborg et al., 2010; Kosek et al., 2018). In total, ten proteins had a significant correlation across CSF and serum and were thus potentially involved in blood borne periphery-to-CNS neuroimmune cross-talk in OA. Contrary to the notion that neuroinflammation is a contributing factor to chronic pain, we demonstrated that 7 CSF inflammatory proteins, CSF-1, CX3CL1, HGF, LIF-R, SCF, TWEAK and VEGFA, were

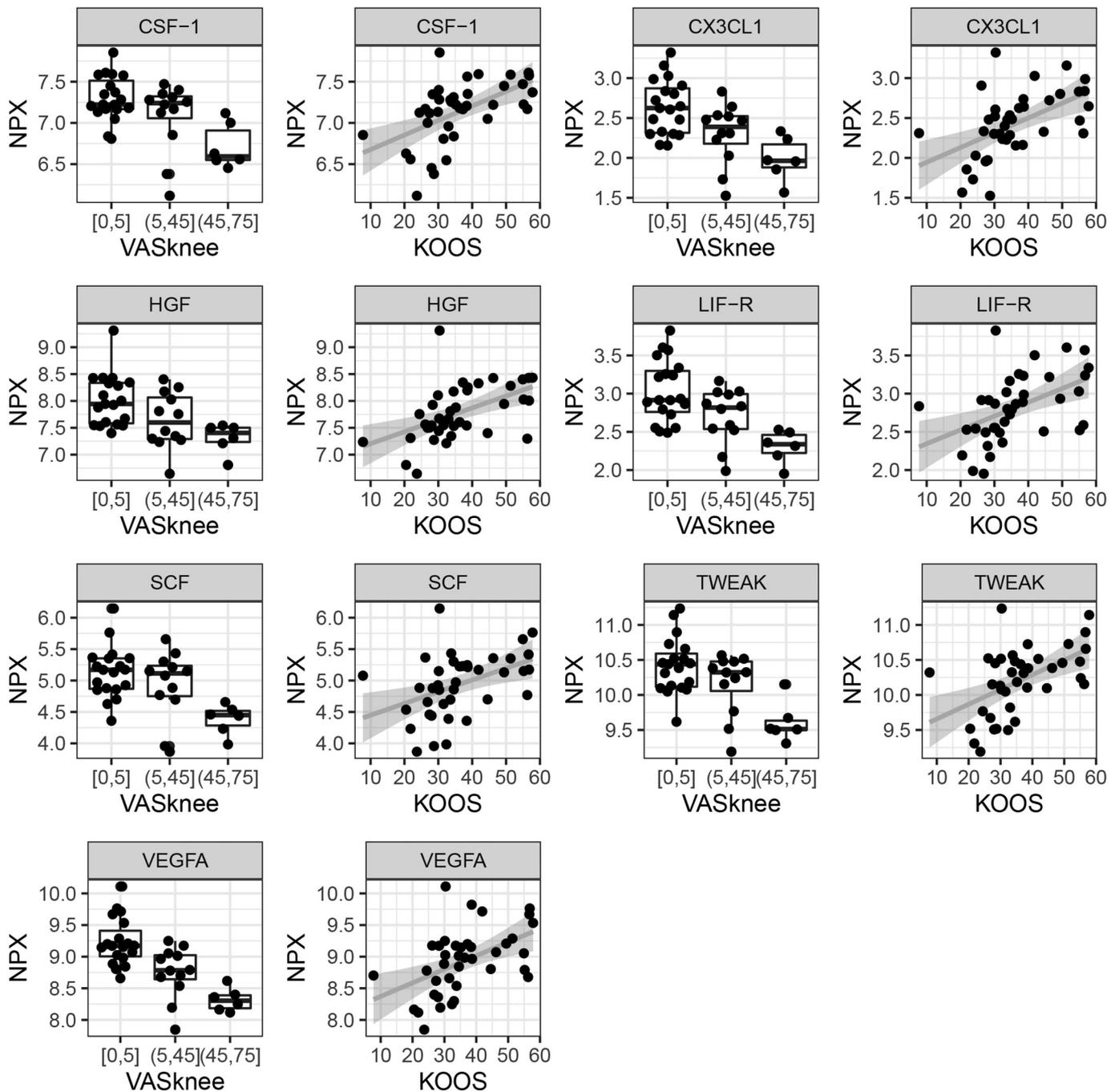


Fig. 2. The associations between the CSF levels of the top proteins to pain ratings (VASknee) and knee-related symptoms (KOOS).

The seven proteins in CSF which were significantly associated with lower intensity of knee pain (lower VASknee) as well as milder knee-related symptoms (higher KOOS ratings) are included. Parametric ANOVA adjusted for age, gender, plate effect and BMI. The FDR corrected p values (q values) were as following: CSF-1: VAS q = 0.011, KOOS q = 0.012, CX3CL1: VAS q = 0.0063, KOOS q = 0.012, HGF: VAS q = 0.023, KOOS q = 0.012, LIF-R: VAS q = 0.0063, KOOS q = 0.015, SCF: VAS q = 0.016, KOOS q = 0.023, TWEAK: VAS q = 0.0063, KOOS q = 0.012 and VEGFA: VAS q = 0.00054, KOOS q = 0.015, please see [Table 4](#) for Df, F and p values. The VASknee values were divided into 3 subgroups: 0 to 5 mm (no pain), 6 to 45 mm (mild pain) and 46 to 75 mm (moderate pain). The FDR adjusted P-values < 0.1 were significant for all the proteins, meaning that the levels between the groups are different (no pairwise testing performed). CSF = cerebrospinal fluid, NPX = Normalized protein expression, VAS = visual analogue scale (0–100; 0 = no pain; 100 = the worst imaginable pain), KOOS = Knee injury and Osteoarthritis Outcome Score (0–100; 0 = the worst symptoms; 100 = no symptoms), CSF-1 = Macrophage colony-stimulating factor 1, CX3CL1 = Fractalkine, HGF = Hepatocyte growth factor, LIF-R = Leukemia inhibitory factor receptor, SCF = Stem cell factor, TWEAK = Fibroblast growth factor 21, VEGF-A = Vascular endothelial growth factor A.

associated with lower pain intensity as well as milder knee related symptoms and all of them had elevated levels in the CSF of patients compared to controls. Interestingly, we did not find any inflammatory proteins in CSF that were related to more severe pain or knee related symptoms and none of the assessed serum proteins were directly

associated with OA symptoms. Our findings suggest that inflammatory proteins exert an analgesic and/or protective role in the CNS of OA patients, but do not support the use of inflammatory proteins in serum as biomarkers for pain. The proteins ADA and IL-18R1 are of particular interest, as they have higher CSF levels in OA patients, a positive

correlation between CSF and serum levels and a negative association with symptom severity.

4.1. Proteins potentially involved in periphery-to-CNS neuroinflammatory cross-talk

The bidirectional cross-talk between the peripheral tissues and the CNS is mediated by the nervous and the immune systems (Svensson et al., 2003; Zhang et al., 2007; Ji et al., 2013; Xanthos and Sandkühler, 2014; Littlejohn, 2015; Wu et al., 2017). Persistent peripheral inflammation can increase the transfer of pro-inflammatory cytokines from the periphery to the CNS across the BBB (Gutierrez et al., 1993; Quan and Herkenham, 2002; Greter et al., 2005; DosSantos et al., 2014) and increase the permeability of the BBB for infiltration of immune cells from the periphery (Reijerkerk et al., 2012; Wu et al., 2017). Circulating cytokines can also activate brain endothelial cells or glia to produce cytokines de novo within the CNS (Watkins and Maier, 2005; Wu et al., 2017). Furthermore, growing evidence suggests that inflammatory mediators released from immune cells invading the dorsal root ganglia can activate the resident glial cells to release pro-inflammatory cytokines and chemokines and thus contribute to neuroinflammation and pain (Zhang et al., 2007; Grace et al., 2014; Verma et al., 2015).

We hypothesized that substances involved in the blood borne periphery-to-CNS neuroimmune communication in OA would be characterized by a positive correlation between their levels in CSF and serum. In the current study, we identified ten substances fulfilling these criteria: ADA, CCL4, CCL11, CCL25, CXCL9, FGF-21, IL-10RB, IL-12B, IL-18R1 and uPA. To our knowledge, at least seven of these have previously been shown to influence BBB permeability. Surprisingly, only three have been linked to increased BBB permeability, namely CCL4 (Quandt and Dorovini-Zis, 2004), CCL11 and IL-12 (Chai et al., 2014), while FGF-21 (Chen et al., 2018) and uPA (Tan et al., 2017) have been reported to decrease BBB permeability. In addition, as we are measuring soluble receptors, i.e., the presence of these receptors indicates neutralization of their ligand, IL-10RB and IL-18R1 could also be associated with decreased BBB permeability as their ligands IL-10 (Lin et al., 2018) and IL-18 (Jung et al., 2012) have been reported to increase BBB integrity. Finally, ADA would be expected to improve BBB integrity by degrading adenosin, as adenosin increases BBB permeability (Carman et al., 2011; Kim and Bynoe, 2015).

Three of these 10 substances have been implicated in the regulation of the migration of lymphocytes across BBB: CCL4 by affecting the adhesion of T-cells to human brain endothelial cells (Quandt and Dorovini-Zis, 2004), CCL25 by promoting lymphocyte infiltration to the brain (Zhang et al., 2019) and CXCL9 by recruiting T cells and monocytes across the BBB (Park et al., 2002). Finally, CCL11 has been shown to be transported across the BBB, without affecting the permeability, and has been proposed to exert a regulatory role in the chemokine system (Erickson et al., 2014).

In conclusion, in accordance with our a priori hypothesis, the majority of the substances with correlating protein levels between CSF and serum have previously been reported to influence the BBB or the recruitment of immune cells to the CNS and are thus likely to be involved in blood borne periphery-to-CNS signaling. Furthermore, compared to controls, OA patients had higher levels of 9 of these proteins (all except IL-12B) in the CSF and the levels of IL-18R1 and FGF-21 were also higher in serum. Finally, the CSF levels of IL-18R1 and ADA were also associated with less pronounced OA symptoms.

4.2. Inflammatory proteins in CSF are associated with less severe OA symptoms

Despite substantial evidence of neuroinflammation in several painful conditions such as neuropathic pain (Kotani et al., 2004; Bäckryd et al., 2017a; Backonja et al., 2008), fibromyalgia (Bäckryd

et al., 2017b; Kadetoff et al., 2012), lumbar disc herniation (Brisby et al., 2002; Palada et al., 2019), rheumatoid arthritis (Lampa et al., 2012; Kosek et al., 2015) and OA (Lundborg et al., 2010; Kosek et al., 2018), the influence of neuroinflammation on pain is not well understood. To our knowledge, only five previous studies assessed the correlation between CSF levels of pro-inflammatory cytokines and pain, two found a positive correlation, namely between IL-1b and pain in patients with peripheral neuropathy (Backonja et al., 2008) and between IL-8 and pain in patients suffering from lumbar disc herniation (Palada et al., 2019), while three did not find any significant associations between cytokines in CSF and ratings of pain intensity in patients suffering from lumbar disc herniation (Brisby et al., 2002), rheumatoid arthritis (Lampa et al., 2012) or hip/knee OA (Lundborg et al., 2010). However, the number of investigated cytokines in the previous studies was small and did not include any of the 7 inflammatory proteins linked to reduced pain and symptom intensity in the present study. In a hypothesis driven study of the current cohort, we previously reported that elevated IL-8 concentrations in CSF were associated with reduced pressure pain sensitivity, whereas CSF IL-6 levels were associated with milder knee related symptoms (lower KOOS) (Kosek et al., 2018). Both findings were reproduced in the current study but did not survive correction for multiple comparisons. Here, we identified seven inflammatory proteins in CSF (CSF-1, CX3CL1, HGF, LIF-R, SCF, TWEAK and VEGFA), associated with less intense knee pain and milder symptom severity (Fig. 2). The CSF levels of all 7 proteins were higher in OA patients than controls and all seven proteins had elevated levels in patients with no or mild pain compared to moderate pain, which indicates that they have analgesic and/or neuroprotective effects.

In fact, neuroprotective effects have previously been reported for all of these substances. VEGFA exerts anticytotoxic effects on neurons (Beazley-Long et al., 2013; Hulse et al., 2014; Hulse, 2017), while SCF and HGF are neurotrophic (Takagi et al., 2008; Kessler et al., 2015). LIF-R confers neuroprotection through upregulation of antioxidant enzymes (Davis and Pennypacker, 2018), whereas TWEAK increases neuronal tolerance to hypoxia (Echeverry et al., 2012) and both substances have anti-inflammatory effects (Wicovsky et al., 2009; Boulamery and Desplat-Jégo, 2017; Davis and Pennypacker, 2018). The molecular function of CX3CL1 is complex and includes anticytotoxic, neurotrophic and antioxidant effects, although neurotoxic effects have also been reported (Nash et al., 2015; Luo et al., 2019). In addition, CX3CL1 and CSF-1 are most likely neuroprotective by enhancing the microglia M2 (alternative, protective phenotype) and reducing the M1 phenotype (classical, pro-inflammatory activation) (Limatola and Ransohoff, 2014; Nash et al., 2015; Kiyota et al., 2018; Luo et al., 2019).

It could be speculated that these neuroprotective mechanisms would counteract the negative impact of nociception on endogenous pain modulation. Increased pain sensitivity, dysfunction of descending pain inhibition (CPM), as well as reduced grey matter volumes in brain regions associated with descending pain modulation have been reported in OA patients and normalized following successful surgical pain relief (Kosek and Ordeberg, 2000a; Kosek and Ordeberg, 2000b; Rodriguez-Raecke et al., 2009; Arendt-Nielsen et al., 2010; Gwilym et al., 2010) indicating that these aberrations were caused and maintained by nociceptive input from the OA joints. However, none of the identified seven substances associated with lower symptom severity were related to pain sensitivity (PPTs) or the function of CPM, suggesting other mechanisms of action.

The situation regarding analgesic effects of the identified substances is complicated. To our knowledge, one has been reported to be antinociceptive (HGF), one to be anti- or pronociceptive (VEGFA), three to be pronociceptive (SCF, CSF-1, CX3CL1), and two have, to our knowledge, not previously been associated with pain (LIF-R, TWEAK). More specifically, intramuscular injection of plasmid construct containing human HGF reduced pain behavior in a mouse chronic constriction nerve injury model and reduced pain in patients with painful diabetic

neuropathy (Kessler et al., 2015; Nho et al., 2018). The VEGFA pre-mRNA can be alternatively spliced into VEGFA_{165a} with pronociceptive and VEGFA_{165b}, with antinociceptive effects, respectively, and the antinociceptive VEGFA isoform 165b prevented the development of neuropathic pain in rodents and has been proposed as a novel target for drug development (Hulse et al., 2014; Hulse et al., 2016; Hulse, 2017). Hypersensitivity has been reported following intrathecal injections of SCF (Takagi et al., 2008), CSF1 (Guan et al., 2016) and CX3CL1 (Wang et al., 2018). In addition, CX3CL1 and CSF1 have been implicated in animal models of neuropathic pain (Souza et al., 2013; Sun et al., 2013; Boakye et al., 2019) and CX3CL1 also with nociceptive pain (Kiyomoto et al., 2013). The analgesic effects of HGF and VEGFA_{165b} are in accordance with our findings. The discrepancies between our findings and previous studies regarding the other substances could be explained by differences between short-term animal pain models and chronic pain and/or by disparities between species or site of action. Finally, in Chinese OA patients, CX3CL1 concentrations in serum and SF were related to more pronounced radiological changes, higher pain intensities and more disability (Zou et al., 2013; Huo et al., 2015). The findings regarding serum were not replicated in our study.

4.3. Methodological considerations

For ethical reasons it was not possible to collect CSF from HC. Therefore, we had to rely on an additional CSF control group consisting of patients suffering from recurrent headaches who cannot be considered as truly healthy subjects despite the absence of known neurological or inflammatory pathology. Furthermore, we cannot exclude that the age difference between OA patients and CSF controls (average 17 years) influenced the group differences. Also, due to high number of substances analyzed and the size of the patient cohort, we refrained from reporting sex differences. The exact mechanisms how identified proteins contribute to neuroimmune cross-talk and pain need to be investigated in appropriate animal models. Finally, the analysis was limited to the 91 substances in the OLINK inflammatory panel and cannot be regarded as a comprehensive analysis of all proteins which could contribute to neuroinflammation, neuroimmune communication and symptom severity in OA patients.

4.4. Conclusions

In summary, our results indicate that neuroinflammation in knee OA patients is a protective mechanism associated with reduced pain and milder knee related symptoms. Furthermore, we identified ten proteins with correlating levels across CSF and serum, thus potentially involved in blood borne periphery-to-CNS neuroimmune signaling. All of these, except for IL-12B, were elevated in the CSF of OA patients and IL-18R1 and ADA also related to reduced symptom severity. No biomarkers associated with severity of OA symptoms were found in serum. We propose that agents acting on neuroinflammation in OA may become promising targets for future drug development.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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