Changes in immune and glial markers in the CSF of patients with Complex Regional Pain Syndrome


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Abstract

Complex Regional Pain Syndrome is a severe chronic pain condition characterized by sensory, autonomic, motor and dystrophic signs and symptoms. The pain in CRPS is continuous, it worsens over time, and it is usually disproportionate to the severity and duration of the inciting event. This study compares cerebrospinal fluid (CSF) levels of pro- and anti-inflammatory cytokines, chemokines and several biochemical factors (glial fibrillary acidic protein (GFAP), the nitric oxide metabolites (nitrate plus nitrite), the excitatory amino acid neurotransmitter glutamate, calcium, total protein and glucose) in patients with CRPS to levels found in patients suffering with other non-painful or painful conditions. The aim of the study is to determine the degree of involvement of glial cells and immune system mediators in the pathophysiology of CRPS. There was no elevation or reduction of a CSF marker that was specific for CRPS patients. However, there were several patterns of markers that could be helpful in both elucidating the mechanisms involved in the disease process and supporting the diagnosis of CRPS. The most common pattern was found in 50% (11 out of 22) of the CRPS patients and consisted of; elevated IL-6, low levels of IL-4 or IL-10, increased GFAP or MCP1 and increases in at least two of the following markers NO metabolites, calcium or glutamate. The results from this and other similar studies may aid in elucidating the mechanisms involved in the pathophysiology of CRPS. A better understanding of these mechanisms may lead to novel treatments for this very severe, life-altering illness.

Keywords: CRPS; Cytokines; Chemokines; Cerebrospinal fluid; Glutamate; GFAP; MCP1; Nitric oxide; Calcium

1. Introduction

Complex Regional Pain Syndrome (CRPS) is a severe chronic pain condition characterized by sensory, autonomic, motor and dystrophic signs and symptoms (Janig and Baron, 2003; Schwartzman et al., 2001). CRPS usually develops following an injury or a surgical procedure. In some cases, CRPS can develop after a minor injury such as a sprain, and in other cases no precipitating event can be identified. The pain in CRPS is continuous, it worsens over time, and it is usually disproportionate to the severity and duration of the inciting event.

Studies in both animals and humans have shown that the types of chronic pain observed in CRPS patients can result from multiple mechanisms that include: changes in the peripheral nervous system (PNS) (Waxman et al., 1999); active processes involving both the PNS and the central nervous system (CNS) (Janig and Baron, 2003; Woolf and Salter, 2000); or from a sickness like response involving interactions between the immune and nervous systems (Marchand et al., 2005; Watkins and Maier, 2005). These mechanisms may cause pain individually or in concert.

Clinical studies have shown that some patients with CRPS respond well to intravenous lidocaine (Toda et al., 2006; Wallace et al., 2000), others to the NMDA receptor antagonist ketamine (Correll et al., 2004; Goldberg et al., 2005) and still other patients to immune modulation with

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The degree of involvement of glial cells and painful conditions. The aim of the study is to determine the levels found in patients suffering with other non-painful or painful conditions. Mast cells, neutrophils and macrophages are activated and recruited to the site of injury (Marchand et al., 2005). Disruption of the blood nerve barrier allows for the invasion of the nerve by fibroblasts, macrophages and Schwann cells (McMahon et al., 2005). These cells produce a variety of pro-inflammatory cytokines and chemokines that have been implicated in the generation of neuropathic pain either via direct sensitization of nociceptors or indirectly by stimulating the release of agents that act on neurons and glia (Abbadie, 2005; Watkins and Maier, 2003). Glial cells, in particular microglia and astrocytes, are the immunocompetent cells in the central nervous system (CNS) and are activated following tissue injury or inflammation (Tsuda et al., 2005). Once activated, glia secrete a number of substances known to excite pain transmission in neurons (Kreutzberg, 1996; Watkins et al., 2001). Activated glia have been shown to be both necessary and sufficient for enhanced nociception (Watkins and Maier, 2003).

This study compares cerebrospinal fluid (CSF) levels of pro- and anti-inflammatory cytokines, chemokines and several biochemical factors (glial fibrillary acidic protein (GFAP), the nitric oxide metabolites (nitrate plus nitrite), the excitatory amino acid neurotransmitter glutamate, calcium, total protein and glucose) in patients afflicted with CRPS to levels found in patients suffering with other non-painful or painful conditions. The aim of the study is to determine the degree of involvement of glial cells and immune system mediators in the pathophysiology of CRPS.

2. Methods

Fifty-three patients participated in this study. All patients were enrolled after giving informed consent as approved by the Drexel University College of Medicine Institutional Review Board (IRB). All of the patients were recruited from the general neurology clinics of Drexel University College of Medicine. Twenty-two patients met all IASP criteria for CRPS (Harden and Bruehl, 2005).

The following tests were performed on the CRPS patients when clinically indicated to rule out rheumatologic and inflammatory conditions; rheumatoid factor (RF), antinuclear antibodies (ANA), extractable nuclear antigen antibodies (SSA and SSB), single stranded DNA antibodies, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and a complete blood count (CBC). Gm1 antibody screen was obtained if an autoimmune etiology was suspected in the clinical presentation.

Thirty-one patients with other neurological conditions were also evaluated. The patients with other neurological conditions included: radiculopathies (n = 13), Amyotrophic Lateral Sclerosis (n = 9), peripheral neuropathies (n = 3), spondylolisthesis (n = 4) and normal pressure hydrocephalus (NPH) with a shunt (n = 2). CSF was not obtained from normal controls (volunteers with no known disease).

Cerebrospinal fluid was collected from patients undergoing either lumbar puncture (n = 51) or intrathecal catheter placement (n = 2) for the treatment or diagnosis of their illness. All of the samples were collected during daylight hours between 8 AM and 2 PM. Following collection, the CSF samples were centrifuged at 3000g for 10 min at 4 °C. The supernatant was then split into 250 μl aliquots, frozen and stored at −75 °C until analysis. Each test was performed from thawed aliquots, which were not re-frozen for further testing.

Cerebrospinal fluid levels of cytokines, chemokines and GFAP were determined by high-sensitivity enzyme linked immunosorbent assay (ELISA). The levels of IL-4 and IL-10 were determined with ELISA kits ( Bender MedSystems, Vienna, Austria) with detection limits of 0.1 and 0.05 pg/ml, respectively. GFAP levels were determined with an ELISA kit (BioVendor, Candler, NC) with a detection limit of 25 ng/ml. Levels of IL-6, IL-8 and MCP1 were determined with ELISA kits (R&D Systems, Minneapolis, MN 55413) with detection limits of 0.039, 3.5 and 5.0 pg/ml, respectively. Levels of nitrate and nitrite were determined by fluorometric assay (Cayman Chemical Company, Ann Arbor, MI) with a detection limit of 0.2 μM. Two to three well ELISA plates each were used to determine the CSF levels of GFAP, nitrate and nitrite. CSF total protein levels were determined colorimetrically in 96 well plates by the Bradford method (Bradford, 1976) using a micro protein kit (Sigma-Aldrich, Saint Louis, MO). Glucose levels were evaluated colorimetrically in 96 well plates with the QuantiChrom glucose assay kit (BioAssay Systems, Hayward, CA). Spectrophotometric assays were performed with a Molecular Devices (Sunnyvale, CA) Spectrmax 190 spectrophotometer. Fluorometric assays were performed with a Molecular Devices (Sunnyvale, CA) Spectrmax Gemini XPS spectrofluorometer. All assays were performed in duplicate according to the manufacturers’ instructions.

Prior to amino acid analysis, the CSF was pretreated by ultrafiltration using Amicon (Millipore) filters with molecular weight cutoffs of 10,000 daltons. Glutamate levels were determined by high-performance liquid chromatography (HPLC) with fluorometric detection after pre-column derivatization with N-acetyl-L-homoserine. Erythrocyte glutathione and erythrocyte dehydro (Hashimoto et al., 1992). The derivatized amino acids were analyzed using an octadecylsilyl (C₁₈) column (Microsorb Short-One, Rainin Instrument Co., Woburn, MA). The column was operated at a constant flow rate of 1.0 ml/min at 30 °C. Mobile phase A was 0.1 M sodium acetate buffer (pH 6.0), containing 7% acetonitrile and 3% tetrahydrofuran, and mobile phase B was 0.1 M sodium acetate buffer containing 47% acetonitrile and 3% tetrahydrofuran. The separation of amino acid derivatives was performed using a gradient from mobile phase A to B over 30 min. The fluorescent amino acid derivative was detected using a Gilson model 121 fluorometer (Gilson Inc., Middleton, WI).

Statistical significance between groups was determined by analysis of variance (ANOVA) using the Tukey–Kramer post-hoc multiple comparison test. The data was considered significantly different if p < 0.05. Calculations were accomplished with the aid of statistical data analysis software, SYSTAT version 11 (SYSTAT Software Inc., Richmond, CA).

3. Results

The number of patients, their age, gender ratio, CSF protein and glucose levels as well as the number that demonstrated pain as part of their symptom complex and their mean visual analog scale (VAS) pain score for all groups are tabulated in Table 1. The CRPS patients (17 females, 5 males) had a mean age of 45.5 years (range 27–67 years); a
mean disease duration of 8.4 years (range 0.5–31 years) and reported mean VAS pain scores of 7.2/10 (range 3–10). The CRPS patient’s pain history and medications are tabulated in the Appendix. The thirty-one patients (20 females, 11 males) in the other disease groups had a mean age of 51.9 years (range 20–79 years). Eighteen of these patients (radiculopathies (n = 13), spondylolisthesis (n = 4) and peripheral neuropathies (n = 1)) reported pain that was chronic and at times severe. These patients reported mean VAS pain scores of 6.7/10 (range 4.5–10). In the remaining thirteen patients (peripheral neuropathies (n = 2), NPH with a shunt (n = 2) and amyotrophic lateral sclerosis (ALS) (n = 9)), chronic pain was not part of their symptom complex. There were no significant differences in CSF protein (F(5,47) = 1.86, p = 0.12) or glucose (F(5,46) = 0.65, p = 0.66) levels between patient groups and their levels were within the normal range for lumbar CSF (Fishman, 1992).

The CSF levels of IL-4, IL-6, IL-8, IL-10 and MCP1 in all patient groups are tabulated in Table 2. The CSF levels of all cytokines and chemokines were evaluated from frozen aliquots for this study except for IL-6 values in 19 of the 53 patients. The CSF IL-6 values for those 19 patients (9 CRPS, 7 radiculopathies, 2 peripheral neuropathies and 1 normal pressure hydrocephalus with a shunt) were from a previous study (Alexander et al., 2005). In that study, we evaluated CSF IL-6 levels in twenty-two CRPS patients, the nine Drexel patients from the previous study and an additional thirteen CRPS patients also from our pain clinics at Drexel University College of Medicine. There was no significant difference in CSF IL-6 levels between the three groups; CRPS (n = 15), CRPS Drexel-1 (n = 9) and CRPS Drexel-2 (n = 13) (F(2,34) = 0.84, p = 0.44). There was also no significant difference (F(1,16) = 0.51, p = 0.49) between the ten controls with other neurological diseases from the previous study and the eight additional patients with the same conditions included in this study.

In agreement with our previous study (Alexander et al., 2005), the twenty-two Drexel patients with CRPS demonstrated significantly greater (F(1,38) = 10.55, p < 0.01) levels of the pro-inflammatory cytokine IL-6 as compared to the radiculopathy, peripheral neuropathy, and NPH groups. The spondylolisthesis group also demonstrated significantly increased (F(1,20) = 8.59, p < 0.01) levels of IL-6 as compared to the radiculopathy, peripheral neuropathy and NPH group. The ALS patients showed elevated IL-6 levels as compared to the radiculopathy, peripheral neuropathy and NPH group but the increase was not statistically significant (F(1,25) = 2.44, p < 0.13).

The CSF levels of IL-4 were low in all patient groups and fell below the sensitivity of the ELISA in all of the ALS patients, nine out of the twenty-two CRPS patients and five out of the twenty-two radiculopathy, spondylolisthesis, peripheral neuropathy and NPH patients. When those samples were removed, the CRPS group demonstrated signifi-
significantly reduced ($F(1,28)=8.95, p<0.01$) levels of the anti-inflammatory cytokine IL-4 as compared to the radiculopathy, spondylolisthesis, peripheral neuropathy and NPH patients ($0.157\pm 0.04$ pg/ml versus $0.277\pm 0.14$ pg/ml).

The CRPS, radiculopathy and spondylolisthesis patients (groups where all patients reported chronic pain) demonstrated much lower CSF IL-10 levels than the peripheral neuropathy, ALS and NPH patients (groups in which most patients reported no chronic pain). When IL-10 levels were compared between patients reporting chronic pain (VAS > 0) and those that did not, the patients reporting chronic pain showed significantly reduced ($F(1,43)=9.3, p<0.01$) values of CSF IL-10 (1.42 pg/ml) versus patients reporting no pain (2.69 pg/ml).

The NPH patients demonstrated lower levels of the chemokine IL-8 when compared to the other patient groups, but probably due to the small number of NPH patients, the difference was not statistically significant ($F(1,49)=1.15, p=0.29$). The CSF levels of the chemokine MCP1 was significantly elevated ($F(1,48)=7.4, p<0.01$) in the ALS patients when compared to the other patient groups.

The CSF levels of GFAP, the nitric oxide metabolites (nitrate plus nitrite), glutamate, and calcium in all patient groups are tabulated in Table 3. CSF levels of GFAP are age-dependent with values increasing as individuals get older (Rosengren et al., 1994). The CSF levels of GFAP were elevated in all of the patient groups in this study as compared to the published range for age-matched neurologically healthy individuals (0.10–0.48 ng/ml) (Anderson et al., 2003; Norgren et al., 2004). The CSF levels for the nitric oxide metabolites (nitrate plus nitrite) were also elevated in most of the patients in this study when compared to the range reported in individuals with no neurological symptoms (0.5–4.5 \mu M) (Yamada et al., 1997; Yumite et al., 2001). The patients in the CRPS group demonstrated the highest CSF levels of the nitric oxide metabolites (nitrate plus nitrite) when compared to all other groups.

With the exception of the NPH patients, the CSF levels for the excitatory amino acid neurotransmitter glutamate were elevated in all of the patient groups in this study when compared to reported average CSF levels in individuals with no neurological symptoms (0.17–0.27 ng/ml) (Larson et al., 2000; Peres et al., 2004; Spreux-Varoquaux et al., 2002). The patients in the CRPS group demonstrated the highest average CSF level of the excitatory amino acid neurotransmitter glutamate when compared to all other groups.

CSF calcium levels were within the normal published range (1.0–1.25 mM) (Fishman, 1992; Joborn et al., 1991) in patients in the peripheral neuropathy and NPH groups. Patients in the CRPS, radiculopathy, ALS and spondylolisthesis groups demonstrated significantly increased levels ($F(1,48)=15.5, p<0.01$) of CSF calcium when compared to the peripheral neuropathy and NPH groups.

### 3.1. Discussion

The pathophysiology of CRPS is not well understood, but the evidence indicates that it includes different biological pathways involved in inflammation and central processing of afferent input. Inflammation, tissue damage or nerve lesions can lead to hypersensitivity and allodynia at the site of injury. In some individuals, the pain persists long after the initiating event has healed. There are a number of mechanisms that attempt to explain the pathophysiology of these chronic pain states. Some of these mechanisms are centered on neuronal sensitization (Ikeda et al., 2006; Woolf and Salter, 2000) and others on neuroimmune interactions and the activation of glial cells (DeLeo and Yezierski, 2001; Marchand et al., 2005; Tsuda et al., 2005; Watkins and Maier, 2005).

Studies in animal models of exaggerated pain demonstrate that following tissue injury or inflammation, glial cells (astrocytes and microglia) become activated (Tsuda et al., 2005). Some of the signaling molecules implicated in glial activation include; MCPI, fractalkine, ATP, pro-inflammatory cytokines, substance P and glutamate (Abbadie, 2005; Abbadie et al., 2003; Inoue, 2006; Klein et al., 1997; Nakajima and Kohsaka, 2001; Nishiyori et al., 1998 and Svensson et al., 2003). Once activated, microglia and astrocytes secrete a number of substances known to excite dorsal horn neurons and influence the establishment and maintenance of neuropathic pain (Watkins and Maier, 2000). These substances include pro-inflammatory cytokines, nitric oxide, excitatory amino acids, prostaglandins and ATP (Abbadie, 2005; Marchand et al., 2005 and Wieseler-Frank et al., 2004).

This study set out to investigate changes in levels of multiple biological markers in the CSF of individuals afflicted with CRPS and in patients suffering with other non-painful or painful conditions. The use of markers combined with the clinical examination is essential in determining the presence or absence of disease and monitoring its response to therapy.

### Table 3

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Nitrate + nitrite (uM)</th>
<th>GFAP (ng/ml)</th>
<th>Glutamate (uM)</th>
<th>Calcium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiculopathies</td>
<td>3.85 ± 0.47</td>
<td>0.758 ± 0.15</td>
<td>0.503 ± 0.11</td>
<td>1.47 ± 0.04</td>
</tr>
<tr>
<td>Peripheral neuropathies</td>
<td>4.68 ± 1.18</td>
<td>1.104 ± 0.37</td>
<td>0.432 ± 0.09</td>
<td>1.06 ± 0.14</td>
</tr>
<tr>
<td>Normal pressure hydrocephalus</td>
<td>4.51 ± 3.22</td>
<td>0.920 ± 0.25</td>
<td>0.173 ± 0.10</td>
<td>1.17 ± 0.19</td>
</tr>
<tr>
<td>Spondylolisthesis</td>
<td>2.45 ± 0.64</td>
<td>0.647 ± 0.26</td>
<td>0.474 ± 0.09</td>
<td>1.53 ± 0.11</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>3.13 ± 0.32</td>
<td>0.849 ± 0.25</td>
<td>0.412 ± 0.12</td>
<td>1.36 ± 0.04</td>
</tr>
<tr>
<td>Complex Regional Pain Syndrome</td>
<td>5.83 ± 1.45</td>
<td>0.887 ± 0.13</td>
<td>0.931 ± 0.30</td>
<td>1.41 ± 0.04</td>
</tr>
</tbody>
</table>

This table lists the average CSF level ± the standard error of the mean of the nitric oxide metabolites (nitrate plus nitrite) in micromoles per liter, GFAP in nanograms per milliliter, glutamate in micromoles per liter and calcium in millimoles per liter.
CRPS patients in this study demonstrated elevated CSF levels of both glutamate and calcium when compared to normal individuals. The release of glutamate and substance P in the spinal cord dorsal horn following inflammation or tissue injury (Hunt and Mantyh, 2001; Willis, 2001) can potentially lead to calcium dependent long term potentiation resulting in hyperalgesia (Ikeda et al., 2006). Increased extracellular calcium has been shown to activate nitric oxide synthase (NOS), which is required for the maintenance of hyperalgesia in animal models of persistent pain (Meller and Gebhart, 1993). In addition, variations in extracellular calcium have also been shown to affect neurotransmitter quantal size as well as the probability of transmitter release at central synapses. This effect is mediated by group I metabotropic glutamate receptors (Hardingham et al., 2006).

We evaluated CSF levels of the pro-inflammatory cytokine IL-6, in order to extend our previous work showing an elevation in CSF IL-6 in many CRPS patients. The control patients for CSF IL-6 levels were individuals with radiculopathies, peripheral neuropathies, and NPH. These patients demonstrated CSF IL-6 levels (n = 18, 1.32 pg/ml) similar to previously reported values in normal human volunteers (n = 24, 1.27 pg/ml) (Steenberg et al., 2006). In agreement with our previous work, the CRPS patients in this study showed significantly (F(1,20) = 8.59, p < 0.01) elevated levels of CSF IL-6 as compared to the control group. The increase in CSF IL-6 was not universal, and was only seen in approximately 60% of the CRPS patients in this study. It is also not specific, as patients with other conditions such as Alzheimer’s and Parkinson’s disease (Blum-Degen et al., 1995), seizures (Lehtimaki et al., 2004), sepsis (Verboon-Maciolek et al., 2006) and spondylolisthesis (this study) show significant increases in CSF IL-6 levels. However, our data do show that the elevation of CSF IL-6 in CRPS is greater than that seen in Alzheimer’s and de novo Parkinson’s disease where neuroinflammation has been proposed as part of the neurodegenerative process (Blum-Degen et al., 1995).

With the exception of the NPH group, the CSF levels of the chemokine IL-8 were elevated in all of the patient groups in this study as compared to published values for normal control volunteers (15.5 pg/ml) (Natelson et al., 2005). The values for IL-8 in the CSF of the CRPS, radiculopathies, peripheral neuropathies, spondylolisthesis and ALS patients were comparable to levels reported in patients with postherpetic neuralgia (PHN) (35 pg/ml) (Kotani et al., 2000). In patients afflicted with PHN, the increase in CSF IL-8 correlates with both the degree of pain and the duration of disease (Kotani et al., 2000). In this study, IL-8 levels did not correlate (r(1,49) = 0.06, p = 0.68) with pain levels (VAS scores) and significant differences were not noted (F(1,49) = 0.29, p = 0.59) in CSF IL-8 levels between patients reporting chronic pain (n = 39, 39.7 pg/ml) and patients reporting no pain (n = 12, 33.7 pg/ml).

In this study, the patients in the ALS group demonstrated significantly greater (F(1,48) = 7.4, p < 0.01) CSF levels of MCP1 as compared to all other patient groups. The CSF levels of MCP1 in the ALS patients in our study (518 pg/ml) are comparable to previously reported values for MCP1 in ALS patients (570 pg/ml) (Wilms et al., 2003). In their study, the CSF level of MCP1 in their control group (tension headaches) was 285 pg/ml, which is much less than the CSF level of MCP1 in all of the groups in our study, suggesting that all of the groups in this study, including the CRPS patients, demonstrate elevated CSF levels of MCP1.

Following injury or inflammation, MCP1 is expressed by both neurons (Zhang and De Koninck, 2006) and glial cells (Babcock et al., 2003). The major source of MCP1 expression comes from microglia and GFAP positive astrocytes (Babcock et al., 2003). GFAP, a protein member of the intermediate filament family, is strongly expressed in activated astrocytes and its level in CSF was also increased in all patient groups in this study when compared to published values for age-matched neurologically healthy individuals (Rosengren et al., 1994; Anderson et al., 2003). Given that activated astrocytes are the source for GFAP and one of the major sources of MCP1, it is not surprising that they were positively correlated in the CSF of all patient groups (r(1,47) = 0.45, p < 0.01), and especially in the CRPS group (r(1,20) = 0.55, p < 0.01) (Fig. 1).

Most of the patients in this study showed elevated levels of nitrate plus nitrite with the patients in the CRPS group demonstrating the greatest elevation. In the CNS, IL-4 and IL-10 are expressed in reactive astrocytes and activated microglia (Hulshof et al., 2002; Park et al., 2005). IL-4 and IL-10 have been shown to inhibit inducible NOS (iNOS) expression resulting in decreased NO synthesis by glial cells.
A reduction of iNOS by IL-4 or IL-10 should result in an inverse correlation between their CSF levels and NO metabolites. There was no correlation between CSF NO metabolites and IL-10 in any of the patient groups ($r(1,42) = 0.06, p = 0.71$). However, with the exception of the CRPS patients the CSF levels of NO metabolites (nitrate plus nitrite) in the study patients were inversely correlated with their CSF IL-4 levels ($r(1,14) = 0.60, p < 0.01$) in contrast to the CRPS patients which did not show such a correlation ($r(1,11) = 0.32, p = 0.29$) (Fig. 2). The lack of correlation between IL-4 and NO metabolites in CRPS patients may be due to the fact that IL-4 levels were not high enough to inhibit iNOS or that NO production in these patients resulted from the induction of other isoforms of NOS. It has been proposed that IL-4 regulates brain inflammation by inducing the death of activated microglia, (Park et al., 2005). The level of IL-4 expression in the CRPS patients may be insufficient to reduce brain inflammation and may be a contributing factor to the mechanisms responsible for the pathophysiology of CRPS.

As a group, the CRPS patients in this study demonstrated elevated CSF levels of IL-6, IL-8, MCP1, GFAP, NO metabolites, glutamate and calcium. It was difficult to establish whether the CRPS patients demonstrated elevated or reduced levels of IL-10 and IL-4 as compared to individuals without neurological diseases, since normative values for these cytokines in the CSF range widely in the literature (Bartosik-Psujek and Stelmasiak, 2005; Natelson et al., 2005; Rota et al., 2006; Stoeck et al., 2005). However, except for the IL-4 levels in the ALS group, the CSF levels of IL-10 and IL-4 in the CRPS patients were the lowest of all of the other disease groups.

There was no elevation or reduction of a CSF marker that was specific to the CRPS patients. However there were several patterns of markers that could be helpful in both elucidating the mechanisms involved in the disease process and supporting the diagnosis of CRPS. The most common pattern was found in 50% (11 out of 22) of the CRPS patients and consisted of: elevated IL-6, low levels of either IL-4 or IL-10, increased GFAP or MCP1 and increases in at least two of the following markers NO metabolites, calcium or glutamate. The second most common pattern was found in 18% (4 out of 22) of the CRPS patients and it consisted of: normal IL-6, low levels of either IL-4 or IL-10, increased GFAP or MCP1 and increases in at least two of the following markers NO metabolites, calcium or glutamate. A third pattern was found in 14% (3 out of 22) of the CRPS patients and it consisted of: increased GFAP or MCP1 and low levels of either IL-4 or IL-10. The remaining CRPS patients showed normal levels of GFAP and MCP1 and demonstrated at least one of the following; elevated IL-6, low levels of the anti-inflammatory cytokines or increases in either NO metabolites, calcium or glutamate.

There were several limitations of this study: (1) We studied a relatively small number of CRPS patients, and a larger sample is needed in order to determine which patterns are relevant to the pathophysiology of the dNSisense; (2) We did not have CSF samples from normal control volunteers and in many cases had to compare CSF levels to previously reported values in healthy individuals. Using values from other studies is limited by the variability seen in the literature for levels of cytokines and chemokines in CSF. However, much of the variability is due to the lack of sensitivity of the methods employed. In this study, we made comparisons of our data to normative values from other studies that used methods with the highest sensitivity available and when possible from the same manufacturer as the ELISA kits we employed; (3) None of the CSF samples were from patients with early CRPS (less than 6 months); (4) We did not have CSF samples from the same individual at different time points in order to match CSF marker patterns with the severity of their symptoms and; (5) More sensitive assays are needed. There were a number of markers (IL-1β, TNF-α and fractalkine) that may have provided additional information, but in most samples their CSF levels were below the level of detection of the available assays.

Our hope is that the data obtained from this and other similar studies may aid in elucidating the mechanisms involved in the pathophysiology of CRPS. A better understanding of these mechanisms may lead to novel treatments for this very severe, life-altering illness.

Acknowledgments

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Appendix A

<table>
<thead>
<tr>
<th>Patient</th>
<th>CRPS history</th>
<th>CRPS symptoms</th>
<th>Medications</th>
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<tbody>
<tr>
<td>CRPS-1</td>
<td>Injuries to the lower back and right hip due to a fall. The pain has spread to the whole body.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, temperature asymmetry, skin color changes, edema, hyperhidrosis, decreased range of motion and weakness, difficulties with movement, sleeping and memory</td>
<td>Narcotics, NSAID, anti-seizure, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-2</td>
<td>The pain started following a brachial plexus traction injury. It initially involved the left shoulder and arm and has now spread to the left leg.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, temperature asymmetry, hyperhidrosis, decreased range of motion and weakness</td>
<td>NSAID, anti-spasmodic, anti-anxiolytic drugs</td>
</tr>
<tr>
<td>CRPS-3</td>
<td>Injury to the chest, left upper extremity, and back due to a fall. The pain has spread to the whole body.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, some areas of hypoesthesia, temperature asymmetry, edema, decreased range of motion and weakness</td>
<td>Narcotics, anti-spasmodic, anti-axiolytic drugs</td>
</tr>
<tr>
<td>CRPS-4</td>
<td>The pain started on the back of the head due to an unknown cause. The pain has spread to the whole body.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, temperature asymmetry, edema, decreased range of motion, dystonia and weakness</td>
<td>NSAID, anti-spasmodics, anti-depressant drugs</td>
</tr>
<tr>
<td>CRPS-5</td>
<td>Injuries to right side following a MVA. The pain has spread to the whole body.</td>
<td>Hyperesthesia, allodynia, temperature asymmetry, skin color changes, edema, hyperhidrosis, decreased range of motion and weakness</td>
<td>Narcotics, anti-seizure, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-6</td>
<td>Injuries to head, neck, and right upper extremity following a MVA. The pain has spread to the left upper extremity.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, skin color changes, edema, decreased range of motion and weakness</td>
<td>Narcotics, anti-seizure, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-7</td>
<td>Injury to brachial and cervical plexuses. The patient also has pain in the lower back and left lower extremity that has spread to the right lower extremity.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, skin color changes, edema, hyperhidrosis, decreased range of motion, skin trophic changes and weakness</td>
<td>NSAID, anti-spasmodic, anti-anxiolytic drugs</td>
</tr>
<tr>
<td>CRPS-8</td>
<td>Pain initially in right hand following surgery. The pain has spread to the whole body.</td>
<td>Hyperesthesia, temperature asymmetry, skin color changes, edema, hyperhidrosis and trophic changes</td>
<td>Narcotics, anti-depressants, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-9</td>
<td>The problem started as radicular pain with an L5/S1 distribution that evolved into CRPS symptoms. The CRPS pain started in the right leg and now involves both legs (right greater than left).</td>
<td>Hyperesthesia, some areas of hypoesthesia, edema, weakness, difficulty initiating movement</td>
<td>Narcotics, anti-seizure, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-10</td>
<td>Injury to the lower back due to improper lifting. The pain has spread to the whole body.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, temperature asymmetry, skin color changes, edema, hyperhidrosis, decreased range of motion, motor dysfunction and hair and skin trophic changes</td>
<td>Narcotics, anti-depressants, anti-spasmocic drugs</td>
</tr>
<tr>
<td>CRPS-11</td>
<td>Injury to the arm following a fall. The pain has spread to the whole body.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, temperature asymmetry, skin color changes, edema, hyperhidrosis, decreased range of motion and motor dysfunction</td>
<td>Narcotics, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-12</td>
<td>Injury due to a fall on ice, the pain involves both upper and lower extremities.</td>
<td>Hyperesthesia, hyperhidrosis of the involved extremities and weakness</td>
<td>Narcotics, anti-seizure drugs</td>
</tr>
<tr>
<td>CRPS-13</td>
<td>Injury to right brachial plexus due to repetitive strain. The pain has spread to the whole body.</td>
<td>Hyperesthesia, allodynia to light touch and pressure, temperature asymmetry, edema, hyperhidrosis, weakness, and skin trophic changes</td>
<td>NSAID, anti-seizure, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-14</td>
<td>Pain in upper extremities due to brachial plexus traction injury. Right side worst than the left.</td>
<td>Hyperesthesia, allodynia to light touch, alldodynia to deep somatic pressure, temperature asymmetry, cold allodynia by QST, weakness and tremors</td>
<td>Narcotics, NSAID, anti-seizure, anti-depressant drugs</td>
</tr>
<tr>
<td>CRPS-15</td>
<td>The pain started in the right arm from overuse syndrome. The pain now involves the left arm.</td>
<td>Hyperesthesia, allodynia to light touch, alldodynia to deep somatic pressure, weakness, skin color changes, weakness, dystonia, difficulty initiating movement, cold allodynia by QST, weakness and tremors, difficulty initiating movement, cognitive deficits and difficulty sleeping</td>
<td>Narcotics, NSAID, anti-seizure drugs</td>
</tr>
<tr>
<td>CRPS-16</td>
<td>Pain initially in right leg following back surgery. The pain now involves the right arm and left leg.</td>
<td>Hyperesthesia, allodynia to light touch, alldodynia to deep somatic and joint pressure, temperature asymmetry, skin color changes, weakness, dystonia, difficulty initiating movement, thinking and difficulty sleeping</td>
<td>Anti-seizure, anti-depressants, anti-spasmodic, anti-anxiolytic drugs</td>
</tr>
<tr>
<td>CRPS-17</td>
<td>The pain started in the back following a MVA. The pain has spread to the whole body.</td>
<td>Hyperesthesia, some areas of hypoesthesia, alldodynia to light touch, alldodynia to deep somatic and joint pressure, skin color changes, weakness, skin trophic changes, decreased motor coordination</td>
<td>Narcotics, NSAID, anti-seizure, anti-depressants, anti-spasmodic drugs and a lidocaine patch</td>
</tr>
<tr>
<td>Patient</td>
<td>CRPS history</td>
<td>CRPS symptoms</td>
<td>Medications</td>
</tr>
<tr>
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<tr>
<td>CRPS-18</td>
<td>The pain started from overuse syndrome of the right arm and now involves the right leg.</td>
<td>Cold and heat hyperalgesia on QST, allodynia to light touch, allodynia to deep somatic and joint pressure, temperature asymmetries, skin color changes, weakness, dystonia, tremor, skin trophic changes, difficulty initiating movement and sleeping</td>
<td>Narcotics, anti-seizure, antidepressants, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-19</td>
<td>The pain started in both upper extremities and neck following surgery for the repair of Arnold Chiari malformation.</td>
<td>Hyperesthesia, allodynia to light touch, allodynia to deep somatic pressure, hyperhidrosis, weakness</td>
<td>Narcotics, NSAID, anti-seizure, antidepressants, anti-spasmodic drugs and a lidocaine patch</td>
</tr>
<tr>
<td>CRPS-20</td>
<td>The pain started in the right ankle following an MS attack and has spread to her whole right side.</td>
<td>Hyperesthesia, allodynia to light touch, allodynia to deep somatic pressure, skin color changes, edema, weakness, tremor, dystonia, movement and cognitive difficulties</td>
<td>Narcotics, anti-spasmodic, anti-anti-inflammatory drugs</td>
</tr>
<tr>
<td>CRPS-21</td>
<td>The pain started in the right knee following a MVA. The pain has spread but is still confined to the knee area.</td>
<td>Hyperesthesia, allodynia to light touch, allodynia to deep somatic pressure, skin color changes, hyperhidrosis, edema, weakness, dystonia, difficulty initiating movement</td>
<td>Narcotics, NSAID, anti-spasmodic drugs</td>
</tr>
<tr>
<td>CRPS-22</td>
<td>The original pain was in the right forearm following neck surgery. The area of pain has spread but is still confined to the right arm.</td>
<td>Allodynia to light touch, allodynia to deep somatic and joint pressure, skin color changes, temperature asymmetry, hyperhidrosis, edema, weakness, dystonia, tremor, difficulty with movement initiation, sleeping and memory</td>
<td>Narcotics, NSAID, anti-seizure, antidepressants, anti-spasmodic drugs</td>
</tr>
</tbody>
</table>

### References


