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Patterns of chronic inflammation in extensively treated patients with arachnoiditis and chronic intractable pain

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ABSTRACT

Objective: To use biomarkers to gain insight into and gauge the residual (post-treatment) level of inflammation in two groups of intensively treated patients with severe chronic pain.

Methods: Three study groups were analyzed, and included: (i) patients (n = 90) with chronic intractable pain (CIP), (ii) patients (n = 26) with chronic pain and MRI-documented arachnoiditis (ARC) and (iii) normal subjects without a diagnosis of chronic pain (n = 86). We determined and compared the serum concentrations of Alpha-1 Antitrypsin (A1AT), Myeloperoxidase (MPO) and soluble Tumor Necrosis Factor receptor type 2 (sTNFR2) in each of the patient populations studied.

Results: Patients treated for ARC or CIP had higher serum levels of A1AT and MPO than normal untreated subjects without a diagnosis of chronic pain. ARC patients had an A1AT mean serum concentration of 167.9 ± 41.9 mg/dL as compared to 148.9 ± 35.2 mg/dL for normal subjects (p = 0.023). CIP patients had the highest mean serum A1AT level 183.6 ± 39.2 mg/dL with p values of <0.0001 or 0.85 when compared to normal subjects or ARC patients respectively. ARC patients had an MPO mean serum concentration of 344.6 ± 227.9 ng/mL as compared to 188.2 ± 107.5 ng/mL for normal subjects (p = < 0.0001). CIP patients had a similar mean serum MPO level of 352.3 ± 164 ng/mL with p values of <0.0001 or 0.85 when compared to normal subjects or ARC patients respectively. In addition, we noted a difference in the pattern of MPO expression in patients with ARC in that 34% had levels of MPO at normal or below and 31% had levels 2-fold or greater than normal.

Conclusion: This data supports the concept that in centralized pain, sites of neuroinflammation elaborate MPO and other inflammatory factors which may not be completely cleared from the system despite extensive and complex treatment regimens.

1. Introduction

Severe chronic pain is one of the most intractable and difficult syndromes to treat successfully. Chronic pain is usually defined as any persistent or intermittent pain that lasts more than 3 months [1,2]. Inflammation and pain usually go hand-in-hand, since tissue injury initiates the inflammatory process resulting in a cascade of biochemical reactions that prime the nervous system for pain sensing [3–6]. Moreover, long-term inflammation reinforces adaptive changes in the nervous system that can cause the sensation of pain to become exaggerated, chronic, and can affect a specific part of the body or encompass multiple regions [7–9]. In particular, there is a subgroup of patients that have a chronic intractable pain (CIP) syndrome or disease, all have incurable, extremely painful conditions, as evidenced by failure of various interventions to control their pain, including surgery, nerve blocks, physical rehabilitation, and some opioids. These patients may be non-functional, bedridden, or housebound and often suffer from fatigue, insomnia, depression, and anxiety. Objective assessment invariably reveals intermittently elevated blood pressure and pulse rate and abnormal concentrations of serum cortisol and other adrenal hormones, indicating the presence of a severe and extended stress state [9–11]. In clinical practice, CIP patients are often considered to have a central pain syndrome when affected individuals have multifocal pain combined with other somatic symptoms indicated earlier.

Arachnoiditis (ARC) is a chronic pain condition caused by an inflammation of the arachnoid lining—one of the three linings that surrounds the brain and spinal cord [12]. While somewhat rare, the most severe cases of ARC are associated with intense lumbar pain. Physiologically, ARC is a nonspecific inflammatory process usually caused by an invasion into the dural sac—whether by bacteria, blood, or injections of various irritant substances that produce a spectrum of pathological changes originating on the arachnoid membrane. The resulting inflammatory response eventually proliferates to other intrathecal neural elements leading to fibrosis and adhesions that involve the nerve roots, the arachnoid, the spinal cord, and the dura mater. This progression culminates in permanent disability characterized by severe intractable pain, neurological deficit, and other related symptoms. While the pathophysiology of ARC and CIP are very different, nonetheless, both ARC and CIP are highly debilitating conditions which require extensive and complex treatment. The goal of such treatment is to improve a patient’s function.
and quality of life by reducing inflammation and alleviating symptoms, especially pain.

The primary objective of this study was to determine if we can gain insight into and gauge the residual (post-treatment) level of inflammation in chronic pain states. While biomarker measurements of inflammatory biomarkers in cerebral spinal fluid may be an interesting investigative tool for neuroinflammation, they are difficult to use in routine patient monitoring—particularly when multiple tests on severely compromised patients are proposed. We have focused our study on inflammatory mediators in the serum of patients, since the eventual application of such measurements to the clinical monitoring of patients with severe pain would be less invasive.

2. Materials and methods

2.1. Pain patient populations and normal subjects

The three study groups analyzed include (a) patients with chronic intractable pain (CIP) from the Veract Intractable Pain Clinic (n = 90), (b) patients with a diagnosis of ARC (n = 26), and (c) a series of prospectively collected sera from subjects without chronic pain. Samples were collected from patients during the years 2012–2016 and were stored at a temperature lower than −80°C prior to assay.

Gender and age distribution of the subjects are given in Table 1. The ARC group was a prospective collection of patients who presented with severe pain and whose MRI confirmed the diagnosis. Twenty ARC patients (77%) were female and six (23%) were male. The average body mass index (BMI) for ARC females was 27.0 ± 4.1 (range 19.0–31.8). ARC males had an average BMI of 26.6 ± 2.7 (range 21.6–2.7).

The CIP study group consisted of 56 (62%) females and 34 (38%) males. The average BMI for females was 26.5 ± 7.8 (range: 13.3–48.7). Males had an average BMI of 27.9 ± 4.8 (range: 19.6–40.9).

There were no specific exclusion criteria for pain patients based upon gender or other comorbidities; patients 18 years and younger were excluded. All medications were allowed; while they represent potential confounders, they were substantially diverse and often used in combination and essential for the patient’s well-being.

Samples from healthy normal subjects (non-pain) were obtained from the Brain Institute of the University of Utah (n = 9) Caritas St. Elisabeth Medical Center (Boston, MA) (n = 3). These 12 patients were selected based upon clinical interviews at both academic sites. A series of serum samples from normal subjects (n = 31) with no history of neurologic disease, mood disorders, or chronic pain were obtained from a commercial source (PrecisionMed, San Diego, CA). In addition, we procured serum samples prospectively from healthy volunteers in both San Diego, CA (n = 9), and Durham, NC (n = 35). Non-pain subjects from local sources were excluded if they were taking drugs for chronic pain (e.g. opioids, NSAIDS, antidepressants). The quality of life in the normal volunteer population was assessed using the PHQ-9 [13]; subjects with PHQ-9 scores >5 were excluded. Female subjects without chronic pain (n = 40) had an average BMI of 25.0 ± 6.4 as compared to males (n = 46) who had an average BMI of 27.7 ± 4.8.

2.2. Sample collection and handling

Each study subject provided a blood sample, which was processed to collect serum. The sites prepared serum under standardized conditions. Briefly, blood was allowed to clot for 30 min, centrifuged 10 min at 1300 × g (relative centrifugal force) to collect serum that was aliquoted within 30 min of centrifugation. Serum samples were promptly shipped at 4°C (or frozen at −80°C until ready for shipment on dry ice) to the Ridge Diagnostics CLIA Laboratory (Research Triangle Park, NC). The date and time of the blood draw was recorded by the study site for each sample, along with subject gender, height, and weight. All samples were identified by a sequentially applied accession number which blinded the technician doing testing to the source of the sample and patient identifiers.

2.3. Serum biomarker assays

Serum levels of alpha-1 antitrypsin (A1AT), myeloperoxidase (MPO), and soluble tumor necrosis factor alpha receptor type II (sTNFR2) were measured by validated individual quantitative immunoassays. Standard curves for calibrating the quantity of each biomarker were generated by non-serial dilution of each purified protein. Normal reference ranges (mean ± 2SD) for each biomarker were previously determined from a large healthy control group; gender-specific differences in normal reference ranges were observed for some analytes. A1AT serum concentrations were measured by an analytically validated immunoturbidimetric assay developed at the Ridge Laboratory. sTNFR2 levels in serum were quantified using ELISA kits (Quantikine, R&D Systems/BioTechnie, Minneapolis, MN). MPO was quantified using an ELISA kit (ALPCO, Salem, NH).

2.4. Calculations

Age, gender, height, and weight of each subject were recorded and BMI was calculated using the formula BMI = (weight in lbs. × 703)/(height in inches)^2 and an online calculator [http://www.mayo clinic.com/health/bmi-calculator/NU00597]. Probability (p values) was calculated using the Student’s t-test to assess the statistical significance of differences in mean serum concentration of each biomarker between the chronic pain populations and healthy subjects without chronic pain.

3. Results

3.1. Chronic inflammatory biomarkers in patients with chronic pain

We measured the serum concentrations of three biomarkers of chronic inflammation: A1AT, MPO, and sTNFR2. Box Whisker plots (Figure 1) show significant variation in serum levels of

Table 1. Patient demographics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Females</th>
<th>Males</th>
<th>Age</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachnoiditis</td>
<td>20</td>
<td>6</td>
<td>47.9 ± 10.2</td>
<td>20–72</td>
</tr>
<tr>
<td>CIP</td>
<td>56</td>
<td>34</td>
<td>48.5 ± 10.4</td>
<td>24–72</td>
</tr>
<tr>
<td>Non-pain</td>
<td>40</td>
<td>46</td>
<td>42.7 ± 13.9</td>
<td>20–72</td>
</tr>
</tbody>
</table>
the inflammatory biomarker A1AT in chronic pain patients and normal subjects. ARC patients had an A1AT mean serum concentration of 167.9 ± 41.9 mg/dL (range 87–263 mg/dL) as compared to 148.9 ± 35.2 mg/dL (range 55–260) for normal subjects ($p = 0.023$). CIP patients had a mean level of 183.6 ± 39.2 mg/dL (range 105–281) with $p$-values of $<0.0001$ or 0.08 when compared to normal subjects or ARC patients, respectively.

ARC patients had a mean serum MPO level of 344.6 ± 227.9 ng/mL (range 94–1245 ng/mL) which was higher than the mean concentration for normal subjects, 188.2 ± 107.5 ng/mL (range 38–567) ($p = < 0.0001$). CIP patients had a mean serum MPO level of 352.3 ± 164 ng/mL (range 105–928) with $p$-values of $<0.0001$ or 0.85 when compared to normal subjects or ARC patients, respectively (Figure 2).

In contrast to A1AT and MPO, ARC patients had serum levels of sTNFR2 which were not statistically different from that of normal subjects. The mean serum level for ARC patients was 2636 ± 719 pg/mL (range 1686–4221 pg/mL) as compared to normal subjects 2548 ± 667 pg/mL (range 1633–5798), $p = 0.56$. CIP patients had a mean sTNFR2 serum concentration of 3128 ± 1203 pg/mL (range 1154–7727), with $p$-values of $<0.0001$ or 0.05 when compared to normal subjects or ARC patients, respectively (Figure 3).

### 3.2. Patterns of chronic inflammatory biomarker expression in patients with ARC

We noted significant variation in the pattern of MPO expression in patients with ARC in that 38% had MPO serum levels 2-fold or greater than normal (Figure 4) and 19.2% had levels of MPO at normal or below (Figure 5). There were also a significant number of ARC patients with intermediate serum levels of MPO shown in Figure 6.

### 4. Discussion

In this study of two pain populations and one normal control group, we gained physiological information on biomarker
expression and patterns in ARC and CIP states. The group of patients with intractable pain we evaluated were those with centralized syndromes wherein sensitization of the pain system occurs that can either be relegated to a specific part of the body or encompass the entire body. Centralized pain is typically constant and may be moderate to severe in intensity. CIP patients, as a group, have been shown to have a more severe, persistent pain that usually fail to respond to non-narcotic analgesics and other treatment measures [9–11]. We also examined chronic inflammatory biomarker expression in ARC patients who had both localized and diffuse pain symptomatology. In this study, we measured and compared the expression of three chronic inflammatory biomarkers in each group of chronic pain patients and in comparison to values determined for normal subjects. Alpha-1 antitrypsin, an acute phase protease inhibitor belonging to the serpin superfamily, protects tissues from elastase and other enzymes released from inflammatory cells [14]. Myeloperoxidase is an inflammatory peroxidase enzyme most abundantly present in neutrophil granulocytes [15,16] and activated microglial cells. Lastly, we measured serum levels of sTNFR2, a circulating form of the receptor for TNF alpha, involved in reducing systemic inflammation by binding to TNF alpha [17].

While there are other clinical tests for inflammation, e.g. measurement of C-reactive protein or erythrocyte sedimentation rate, they are generally non-specific—particularly with regard to chronic inflammation [18,19]. The highest mean serum concentration of A1AT, MPO, and sTNFR2 were seen in CIP patients, while A1AT and MPO were significantly elevated in ARC patients as compared with normal subjects. All the patients with chronic pain we studied were being treated, often with a variety of medications both non-narcotic and narcotic. Our observation that subsets of the three inflammatory biomarkers can be expressed in these aggressively treated patients suggests that despite treatment residual inflammation is present. A1AT and sTNFR2 are anti-inflammatory mediators which circulate systemically at reasonable concentration to block pro-inflammatory molecules released by inflammatory cells [14,18]. It is important to note that the serum levels of each of the individual inflammatory biomarkers varies to the extent that no cut-off point can be identified for a single individual biomarker that would segregate normal subjects from pain patients or ARC from CIP.

Interestingly, MPO was the statistically predominant inflammatory marker elevated above normal in treated patients with centralized pain. We hypothesize that such activated microglial cells directly contribute to inflammation. MPO’s activity is associated with powerful pro-oxidative and proinflammatory properties [16,17]. Its role in the establishment and maintenance of neurodegenerative disease states may be related to the elaboration of MPO by microglial cells [20–23]. Although substantial evidence has established that microglia and astrocytes play a key role in the establishment and maintenance of persistent pain in animal models [24,25], the role of glial cells in human pain disorders has not been well established.

We have suggested that in CIP and ARC, microglial cells elaborate MPO and other inflammatory factors which may not be completely cleared from the system despite extensive and complex treatment regimens.

Clearly the size of the ARC group was smaller than we would have liked, and while we identified different patterns of residual inflammation in ARC patients (Figures 4–6), larger groups of well characterized patients would need to be evaluated to see if differences in the clinical manifestation and/or prognosis can be related to specific patterns of biomarker expression. Similarly, we need to follow up on preliminary studies which have indicated that patterns of inflammatory biomarkers may change upon therapy.

It should be emphasized that we have focused upon mean serum biomarker concentrations. As shown in Figures 1–3, within each group, serum biomarker levels for individual patients can vary across a broad range of concentration.
Given the inter-patient variation, it is difficult to segregate CIP patients from patients with ARC or other types of chronic pain based upon fixed cut-off points.

5. Conclusion
The use of biomarkers of chronic inflammatory processes can provide better clinical evidence for continued inflammation and tissue damage. In conclusion, we suggest that monitoring the pre- and post-treatment levels of inflammatory biomarkers and alleviating the source of residual inflammation may be of greater importance than palliative care in treating chronic pain patients.

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Declaration of interest
JA Bilello is a former employee of Ridge Diagnostics Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References