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Neuropathic Pain in Multiple Sclerosis

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Title: Neuropathic Pain in Multiple Sclerosis

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Introduction

Multiple sclerosis (MS) is the most common autoimmune disease of the central nervous system and is characterized by the destruction of myelin, an insulating lipo-protein layer that forms a sheath around neurons to enhance nervous system signaling (3). Currently, an estimated 2.3 million people worldwide are diagnosed with MS and a significant percentage experiences chronic, severe neuropathic pain, with some systematic reviews suggesting that up to 50 percent of MS patients experience chronic pain (2,18). Although the exact mechanisms behind this common and often disabling symptom of MS are still being investigated, discoveries made by our collaborators and other scientific groups are advancing our understanding of MS pathology and the chronic neuropathic pain that can ensue.

It is currently thought that the MS-induced neuropathic pain cascade begins with the upregulation of the metalloproteinase (MMP) family, enzymes that appropriately degrade and turnover biological tissues through their hallmark ability to catalyze biochemical reactions using metals like zinc to perform proteolysis. One key family member, MMP-9, is highlighted in the literature as the principal protein enhanced in the cellular milieu when the peripheral nervous system is damaged (6,7, 13). It is believed that upregulated MMP-9 proceeds to target neuronal myelin and in doing so degrades myelin basic protein (MBP), a fundamental component responsible for the integrity of the myelin sheath.

While MMP-9 Induced MBP proteolysis characterizes initial nerve injury, the transition to neuropathic pain occurs when this proteolysis unmasks a novel peptide epitope (85-ENPVVHFFKNIVTPRTPPPSQ-105) originating from the full-length MBP (6,7,10,17). Previous studies demonstrate that this unmasked myelin basic protein cryptic peptide (MBPCP) plays a direct role in inducing neuropathic pain. Specifically, when Investigators performed intra-neural injections of a synthetic peptide homologous to MBPCP as a one time dose into murine sciatic nerves, animals experienced significantly increased mechanical allodynia after one day when compared to sciatic nerves injected with either a scrambled epitope or mutant epitope (7,13,17,22).

Despite this catalytic microenvironment, the mechanism behind MS related neuropathic pain may have limited contributions from a neuro-inflammatory process as studies characterizing inflammatory cytokine expression in the MS cellular milieu are unremarkable (17). Instead, this proteolysis appears to induce an immunologic process as MBPCP avidly binds to a specific major histocompatibility complex member, HLA-DR2, the human leukocyte antigen most commonly associated with MS (34). The MBPCP-MHC interaction can be further identified and

localized on immune antigen presenting cell surfaces in MS murine model experiments (15). Considering that the presence of MBPCP causes a rise in both helper T-cells and MHCII-reactive cell populations, this novel protein fragment may represent one critical self-antigen that sensitizes and subverts the adaptive immune response to then produce a significant amount of Anti-MBPCP-antibodies, an event ultimately responsible for producing neuropathic pain (31).

It is currently thought that the production of these immunoglobulins recognize and bind with the MBPCP self antigen, forming novel antigen-immunoglobulin complexes that then activate Fc-gamma Receptor 1 (Fc γ RI) on Schwann/neuronal cells from the peripheral nervous system to directly stimulate pain signaling (19,20,21,26). When other animal studies disrupted this paradigm by using intravenous immunoglobulin to block Fc γ RI, animal subjects experienced attenuation in pain during behavioral testing (11). Similarly, performing intraneural injections of synthetic MBPCP in T cell deficient, athymic nude rats demonstrated significantly reduced mechanical allodynia when compared to their wild type counterparts, strengthening the theory that the subversion of the adaptive immune response is crucial to pain pathogenesis as the resulting rise of anti-MBPCP antibody directly stimulates neuropathic pain signaling in animal models (17).

Given the promising characterization of the MMP-9, MBP, and Anti-MBPCP immunoglobulin axis in animal studies modeling multiple sclerosis, this report aims to examine whether this neuropathic process is similar in human studies of multiple sclerosis. This is the first clinical study to explore the possibility of quantifying novel biomarkers to characterize the pain profile of patients with painful conditions such as in MS. In this report, we are able to demonstrate that patients with clinically diagnosed MS do indeed have an increase in anti-MBPCP IgM and IgG in their serum and that the same patients have increased pain profiles when compared to the healthy volunteer control. While our study is currently limited by the number of patients participating, we suspect that as patient enrollment increases in the study, we will further power the statistical trends observed so far and also address the additional question of whether differential biomarker levels can be appreciated when comparing MS patients with chronic pain (defined in the study as a patient reporting an average numeric daily pain rating score ≥ 7 over a month) as compared to patients without chronic pain (defined as an average numeric rating scale pain score ≤ 3 over a month). Based on our results, we believe Anti-MBPCP immunoglobulins may represent one biomarker of clinical neuropathic pain in MS. Ultimately, exploring the relationship between the MMP-9, MBP, and Anti-MBPCP auto-antibodies and MS-associated clinical pain will provide novel diagnostic and therapeutic targets.

Methods

Patient Enrollment

A total of 45 female subjects between the ages of 18-65 need to be recruited for completion of this study. Study participants with confounding psychiatric or pathologic causes of chronic pain were excluded from the study. At this point in time, there have been 6 study participants enrolled in this pilot clinical study. This report summarizes data from 3 patients with MS with chronic pain (defined in the study as a patient reporting an average numeric daily pain rating score ≥ 7 over a month), 2 patients with MS without chronic pain (defined as an average numeric rating scale pain score ≤ 3 over a month), and one healthy volunteer. The original study design specifically required 20 patients with MS with chronic pain, 20 patients with MS without severe chronic pain, and 5 healthy volunteers in order to complete this pilot clinical study with enough statistical power. We are still actively enrolling patients into the study. Patients with multiple sclerosis presented for medical care at either the Multiple Sclerosis clinic or the Pain Medicine clinic at UCSD and were informed about the study through the use of information flyers posted in these specific clinics or by direct communication with their treating physicians and practitioners. Healthy volunteers were recruited from flyers posted on the UCSD campus. Interested study participants were contacted by research staff for more information and screening.

Patient Overview of Expectations

This study required patients to attend a one-time 2-3 hour study visit at the UCSD Clinical and Translational Research Institute. Research coordinators verified each participant's inclusion and exclusion criteria, reviewed informed consent, obtained signed HIPPA forms, and explained the risk, benefits, and alternatives of the study. All study participants then proceeded to history, physical exam, provocative sensory testing, and biologic specimen collection as outlined below. At the conclusion of the study visit participants were compensated \$100 for participating in the study.

Medical History and Physical Exam

The medical history consisted of a focused review of the participant's Multiple Sclerosis symptom course, treatment history, and pain history including a Numeric Rating Scale (NRS), a subjective quantification tool in which patients were asked to rate their average pain level out of ten for the past month. Additionally, a general physical exam assessing all major organ systems was performed to document patient's global physical health. A focused and thorough neurologic physical exam was emphasized to better characterize any physical symptoms of multiple sclerosis including documenting each patient's Kurtzke Expanded Disability Status Scale (EDSS), a tool developed to classify MS patients' functional status (16).

Quantitative Sensory Exam Testing

If MS patients complained of a focal area affected by chronic pain, all sensory testing was performed in that area. In MS patients without a focal area affected by chronic pain, all sensory testing was performed on the lower extremity of the dominant side. A total of five sensory tests were performed:

1. Dynamic Mechanical Allodynia: Superficial application of a 1in foam brush will be applied for a total of three trials. Reported pain intensity was quantified with the use of visual analogue scale (VAS), 0-100mm. Results from each individual trial were averaged to produce the final mean.
2. Static Mechanical Allodynia: Superficial application of a 5.18 von Frey hair for 3 seconds will be applied for a total three trials. Reported pain intensity was quantified with the use of visual analogue pain scale (VAS), 0-100mm. Results from each individual trial will be averaged to produce the final mean.
3. Thermal Pain Intensity: A TSA-II Neurosensory Analyzer from Medoc Advanced Medical Systems applied a 45°C superficial stimulus via temperature probe for a total of three trials. Reported pain intensity was quantified with the use of visual analogue pain scale (VAS), 0-100mm. Results from each individual trial will be averaged to produce the final mean.
- 4: Thermal Pain Threshold: A TSA-II Neurosensory Analyzer machine from Medoc Advanced Medical Systems initially provided a 32°C superficial stimulus via temperature probe. Temperature then increased by 1.5°C/second up to a maximum of 50°C. If thermal pain was experienced prior to 50°C, patients pressed a button that both recorded that threshold temperature and decreased the temperature probe back down to 32°C. A total of three trials were recorded and threshold temperatures were averaged to produce the final mean.
- 5: Mechanical Pain Threshold: Superficial application of a 1cm Wagner FPK manual hand-held pressure algometer determined the minimum force required to produce mechanical pain for a total of three trials. The algometer's reported results from each trial will be averaged to produce the final mean.

Biologic Specimen Collection

The following biologic specimens were collected from each study participant. Samples were prepared, aliquoted into 1ml eppendorf tubes, and frozen at -70°C to be later processed at either UCSD or Sanford-Burnham Prebys Medical Discovery Institute with our collaborating research labs.

1. 10 ml of blood (obtained through venipuncture)
2. 10 ml of urine (obtained through urinary self-collection container)

ELISA Quantification of Anti-MBPCP IgG and IgM antibodies in Participant Blood

For specific details regarding the ELISA protocol for quantifying anti-MBPCP IgG and IgM antibodies in patient serum samples, please refer to the methodology described by Dr. Strongin and Dr. Shubayev (27). In brief, 96-well Maxisorp ELISA plates were coated for 18h at 4°C With ExtrAvidin. Plates were then coated with 1% IgG and Protease-Free BSA suspended in a Tris-HCL buffer for 1h at 37°C in order to block non-specific binding. Plates were then washed with TBS/T for a total of 25 mins at 500-700 RPM with a table-top shaker at ambient temperature. Next either Biotin-labeled WT or Scrambled MBPCP was added to the wells and incubated at 4°C for a total of 16-18h. Plates were then washed with TBS/T for a total of 30 mins at 500-700 RPM with a table-top shaker at ambient temperature. Human serum samples were then diluted 1:50 and then allowed to bind to the MBPCP coated wells for 3h. Plates were then washed with TBS/T for a total of 30 mins at 500-700 RPM with a table-top shaker at ambient temperature before secondary HRP-conjugated human specific IgG or IgM antibodies

were added to the wells in a 1:10,000 dilution. Plates were then washed with TBS/T for a total of 30 mins at 500-700 RPM with a table-top shaker at ambient temperature before adding TMB/E substrate was added. A_{450} values were measured using a plate reader. Experiments were performed three separate times in triplicate.

Statistical Analysis

De-identified clinical data were averaged for each of the three groups including MS with chronic pain, MS without chronic pain, and healthy volunteers. Considering the limited enrollment of participants, we also averaged clinical data for patients with MS as compared to healthy volunteers to look at data trends. Biochemical experiments used a two-tailed unpaired Student's t-test for comparing two groups, or analyses of variance when comparing three or more groups followed by the Bonferroni post-hoc test with $P < 0.05$ for significance.

Results

Patients with MS exhibit history, physical exam, and sensory testing consistent with an elevated pain profile: A total of 5 patients were enrolled in the study at the time of this report (three patients were categorized as MS with chronic neuropathic pain (CNP) and 2 were categorized as MS without chronic neuropathic pain). Additionally, one healthy volunteer with no neuropathic pain was enrolled to compare against. The documented history, physical, and sensory tests are outlined in Figure 1. 100 percent of the patients diagnosed with MS experienced at least 2/6 painful sensations and 100 percent of the patients diagnosed with MS + CNP experienced at least 3/6 painful sensations. When compared to the healthy volunteer who demonstrated no painful sensations, our patient selection demonstrates that patients with MS do experience increased painful sensations. Additionally 1/5 of the patients diagnosed with MS exhibited abnormalities on neurological examination deficit on neurologic exam as compared to the healthy volunteer who had a completely normal physical exam, suggesting the predominance of sensory symptoms and minimal motor symptoms. Finally, 100 percent of the patients with MS reported increased pain sensitivity to at least 1/5 sensory pain tests when compared against the healthy volunteer as a baseline. However, it is important to note that patient M3 did report disproportionately high sensory pain test scores in the absence of other historical or physical signs of neuropathic pain, which could skew any subsequent pooled data analysis.

Patient Demographics						
Patient ID	C1	M1	M2	M3	M4	M5
Group	Control	MS + CNP	MS + CNP	MS - CNP	MS - CNP	MS + CNP
Gender	Female	Female	Female	Female	Female	Female
Age, years	57	64	48	28	34	42
MS Duration, years	0	28	16	8	4	12
Daily Pain Severity Score, #/10	0	10	8	0	0	7
MS Specific Sensory History						
Wetness Sensation						
Burning Sensation		x	x	x		x
Numbness Sensation		x				x
Tingling Sensation		x	x		x	x
Electric Sensation		x	x	x	x	
Trigeminal Neuropathy Sensation						
MS Specific Neurologic Physical Exam						
Kurtzke EDSS, #/10	0	6.5	0	0	0	0
Cranial Nerves II-XII Abnormality						
Gross Sensory Abnormality						x
Muscle Strength Abnormality		x				x
Muscle tone Abnormality		x				
Deep Tendon Reflex Abnormality		x				
Proprioception/Vibration Abnormality		x				
Coordination		x				
Sensory Pain Testing						
Dynamic Mechanical Allodynia: Foam Brush Mean, 0-100	0	0	0	0	0	0.3
Static Mechanical Allodynia: Von Frey 5.18 Mean, 0-100	0	8	17.5	15.2	0	1.6
Thermal Pain Intensity: QST 45 C Pain Mean, 0-100	0	0	0	59.7	0	1.6
Thermal Pain Threshold: QST Temp Mean, C	47.9	50	46.9	47.9	47.7	49.3
Pressure Pain Threshold: Algometer Mean, lb	11.6	14.6	6.7	11.8	7.3	4.2

Figure 1: Summary of pertinent demographic, history, physical, and sensory testing for each individual study participant. Red columns represent MS patients with chronic pain, Yellow columns represent MS patients without chronic pain, and the Green column represents a healthy volunteer. CNP (Chronic Neuropathic Pain)

Patients with MS display elevated Anti-MBPCP IgM and IgG in serum samples: At the conclusion of each patient encounter, blood samples were collected and provided to our scientific collaborators at UCSD or at the Sanford Burnham Prebys Medical Discovery Institute. Each biologic specimen was processed to then provide a high quality serum that underwent ELISA in order to quantify the levels of circulating Anti-MBPCP IgM and IgG. Figure 2 quantifies the A₄₅₀ results from the ELISA and also calculates the fold difference observed against the scrambled peptide as a control. There is the 16.6-fold average difference in circulating Anti-MBPCP IgG between the five MS patients. A smaller 3.6-fold average difference in circulating Anti-MBPCP IgM is also noted for the MS group. While patient M1, M2, M3, M4, all indicate an elevated level of circulating IgG, patient M5 is notable as an outlier considering the insignificant difference between the A₄₅₀ value when compared to the healthy volunteer.

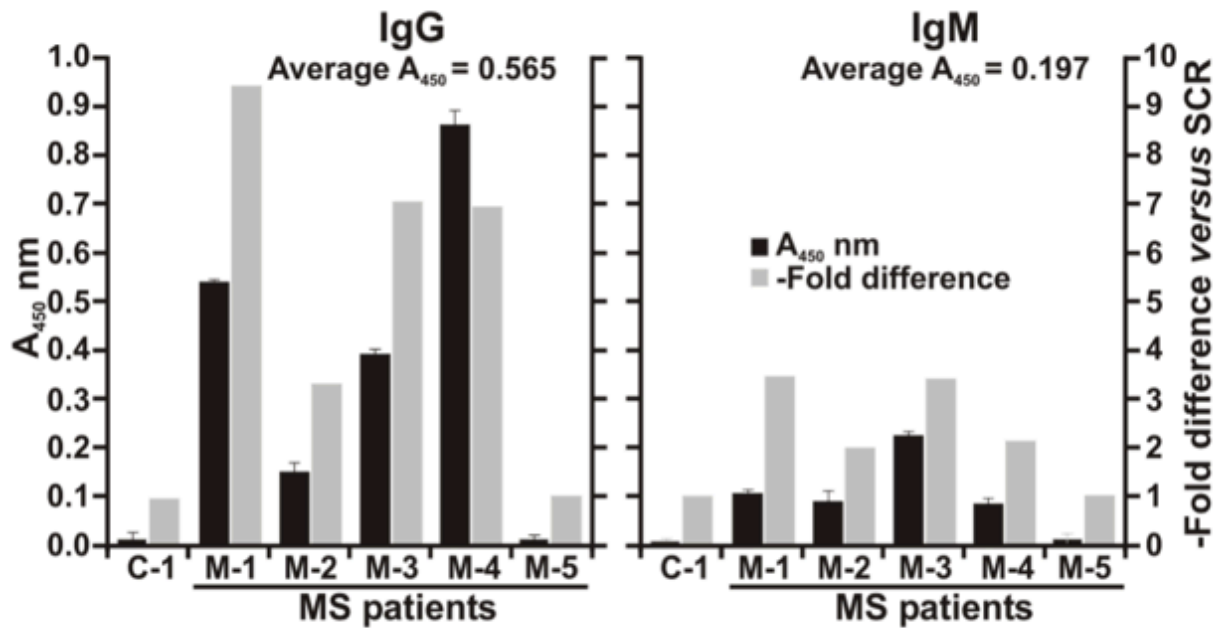


Figure 2: ELISA results quantifying both the circulating IgM and IgG Anti-MBPCP anti-bodies. Data are means \pm SE from three individual experiments performed in triplicate. Black rectangles represent the specific A₄₅₀ values for the WT peptide calculated relative to the SCR peptide. Grey rectangles represent the fold difference against the WT peptide, respectively.

Pooled MS patient data show relationship between average fold difference in Anti-MBPCP levels and clinical sensitivity to pain, however, patient enrollment currently limits further sub-group analysis: Considering that there is a 16.6-fold average difference in IgG and 3.6-fold average difference in IgM in the serum of patients with MS, we expected to see a corresponding increase in the pain profile of patients with MS. When we pool the sensory pain testing results (chosen as this is the most objective measure in the study) from patients M1-M5 we see that 4/5 of the tests show that patients displayed increased sensitivity to pain when compared to the healthy volunteer (Figure 3A). Overall, the pooled analysis reveals that MS patients show an increased sensitivity to pain. Although the small sample size limits proper subgroup analysis, preliminary subgroup analysis was performed to assess whether patients with MS and CNP displayed increased clinical pain and immunoglobulin levels relative to patients with MS and no CNP there currently appears to be no significant relationship. Counter to what we would expect, patients with MS and CNP appear to have a superior sensory pain testing in 4/5 tests as compared to MS patients without CNP (Figure 3B). It is relevant to note that with 3 patients enrolled in the MS and CNP group and 2 patients in the MS without CNP group, these observations are made at a time when the study is still underpowered are highly influenced by extremes in data collected.

(A)

Pooled Patient Demographics		
Patient ID	Control (n=1)	All MS (n=5)
Age Mean, years	57	43.2
MS Duration Mean, years	N/A	13.6
Daily Pain Severity Score Mean, years	N/A	1.3
Kurtzke EDSS Mean	N/A	
Sensory Pain Testing		
Dynamic Mechanical Allodynia: Pooled Foam Brush Mean, 0-100	0	0.06
Static Mechanical Allodynia: Pooled Von Frey 5.18 Mean, 0-100	0	17.36
Thermal Pain Intensity: Pooled QST 45C Pain Mean, 0-100	0	3.36
Thermal Pain Threshold: Pooled QST Temp Mean, Degrees C	47.9	48.36
Pressure Pain Threshold: Pooled Algometer	11.6	9.92

(B)

Pooled Patient Demographics		
Patient ID	MS + CNP (n=3)	MS - CNP (n=2)
Age Mean, years	56	35
MS Duration Mean, years	22	10
Daily Pain Severity Score Mean, years	9	3.5
Kurtzke EDSS Mean	3.25	0
Sensory Pain Testing		
Dynamic Mechanical Allodynia: Pooled Foam Brush Mean, 0-100	0	0.15
Static Mechanical Allodynia: Pooled Von Frey 5.18 Mean, 0-100	12.75	30.65
Thermal Pain Intensity: Pooled QST 45C Pain Mean, 0-100	0	8.4
Thermal Pain Threshold: Pooled QST Temp Mean, Degrees C	48.45	48.6
Pressure Pain Threshold: Pooled Algometer	10.65	10.5

Figure 3: Summary of pertinent demographic, history, physical, and sensory exam testing for each pooled group. Maroon represents all pooled MS patients, Red represents pooled MS patients with chronic pain, Yellow represents pooled MS patients without chronic pain, and Green represents a healthy volunteer.

Discussion

Our current study report aims to address the following two questions. First, do MS patients with neuropathic pain have a significant elevation of MMP-9, MBPCP, anti-MBPCP antibody in collected biologic specimens? Second, is there a relationship between the severity of pain experienced by patients with MS and the previously mentioned biomarker candidates? Our clinical data so far suggests that 5 patients with MS do indeed suffer from an increased pain experience as demonstrated by subjective symptom reports and by the elevated pooled average scores for five provocative sensory pain tests. When pooled, these same 5 patients exhibit an increase in anti-MBPCP IgG and IgM circulating in patient serum. Given the small sample size, it is unclear whether there is any direct relationship between the severity of pain experienced by patients with MS and the quantifiable level of anti-MBPCP IgG and IgM. As illustrated by Figure 3B with patient M-3, extremes in reportable pain levels can cause outlier data that drastically skew the pooled average scores in a small sample size. By increasing the sample size, the effect of these outlier data points will be minimized and any underlying trend can then be appreciated.

Consequently, the greatest limitation to the project is patient enrollment. To appropriately power the study, we originally proposed a total recruitment of 45 patients (20 in the MS with CNP group, 20 in the MS without CNP group, and 5 healthy volunteers as control group). Our approved IRB states that patients will be recruited through the use of informational flyers posted in the Multiple Sclerosis Clinics and Pain Clinics at UCSD. To address this issue we are now considering the following three improvements: amending our IRB recruitment process, adjusting our patient inclusion criteria to include men, and expanding our study group of interest to other forms of neuropathic pain.

Amending IRB Recruitment Process: In order to increase patient recruitment, several possible adjustments can be amended in the IRB. First, our referral base could be expanded by posting the same informational flyers at other health systems in San Diego to collaborate in a multi-center study. For example, Scripps Health, a prominent community health system in San Diego has a separate multiple sclerosis clinic and integrative pain medicine clinic that would represent another avenue of patient recruitment. Similarly, our referral centers could extend beyond San Diego and we could reach out to other academic centers in Southern California including University of Southern California, Loma Linda University and the University of California campuses at Los Angeles, Riverside, Irvine as each of these institutions run their own multiple sclerosis clinic respectively. By pursuing a multi-center collaboration we would dramatically increase our patient recruitment pool but this process would require IRB amendment approval. A final change moving forward would be to rely less on office informational flyers for patient recruitment and to have a research team member attend multiple sclerosis clinics to provide a more personalized approach to patient recruitment. Having a medical student or research assistant partner with the nursing team would allow for the integration of both patient discharge and patient enrollment for interested participants once they have been examined during their scheduled MS follow up visits with a physician.

Inclusion of men with MS in the study: Additionally, another approach to increase patient recruitment could be to adjust the inclusion criteria for the existing study design. Currently our study requires that participants be female with MS but our study can also consider including male participants with MS. This criteria was originally based off the observation that autoimmune disease like MS showed a significant predominance in female populations when compared to male populations with groups reporting anywhere from 2.3-3.5:1 prevalence ratio of women to men diagnosed with MS (1, 8, 24, 33). Although the exact mechanism underlying this specific gender preference is not known, some theories postulate that females have an underlying difference in their immune function with immunological processes that allow enhanced T-cell priming and induction of autoimmunity (29,31). Interestingly, despite the increased frequency of MS in women, other studies found that reportable symptoms are often more severe with a faster disease progression course in men (4). It is currently thought that increased levels of estrogen may improve MS pathology due to a neuroprotective and or immunomodulatory effect (9) and a pilot clinical study treating MS patients with 8mg of daily estrogen have already reported promising immune modulations and decreased MS lesions as evidenced by MRI scans (28). If true, men diagnosed with MS would have the most appreciable effect size difference with respect to symptom profiles and pathologic progression and they should therefore be included in the study.

Expanding our study group to include radiculopathic pain: While the MMP-9, MBPCP, and Anti-MBPCP immunoglobulin pathogenic cascade was originally described for multiple sclerosis, there is a growing body of evidence suggesting that this mechanism may also characterize other processes involving demyelination and consequent neuropathic development (6, 10, 14, 16, 30). Most recently, our collaborators demonstrated that when rat models were subject to unilateral sciatic nerve exposure and had three loosely constrictive chronic gut ligatures surgically placed, a model of trauma-induced chronic nerve injury was produced as mechanical allodynia could be appreciated with hind-paw withdrawal testing 28 days postoperatively. Most interestingly, their body of work demonstrated that animal urine samples tested with urine gelatin zymography displayed an increase of MMP levels and that animal serum samples tested with an ELISA had a 7-fold increase of anti-MBPCP IgM at 28 days as compared to day 0 of nerve injury. This work suggests that the MMP, MBPCP, and Anti-MBPCP immunoglobulin axis is a pathologic process that mediates other myelin-destructive processes besides MS including both traumatic and degenerative events (27). Given the promising results from the animal studies modeling chronic constriction injury, we could expand our clinical pilot study to include any patients with painful radiculopathy. Common conditions such as spinal stenosis or foraminal stenosis may mimic the chronic constriction injuries studied by our collaborators and these patients may also demonstrate a relationship between clinical pain and these novel biomarkers.

Beyond patient recruitment, another limitation with clinical trials studying pain is the reproducibility of the pain testing since self-reported pain scores are inherently subjective. Since patients in this trial displayed variability within their own triplicate testing of sensory exams, this variance could cause outlier data points that would obscure any underlying trends. To reduce the impact of human error in self-reported scores, increasing sensory testing from

three recordings to five recordings and then removing the lowest and highest values collected could reduce self-reported error and allow patients to get used to testing conditions before giving a reproducible pain report.

Finally, one other limitation to consider is that this study uses a single numeric rating system to quantify central pain. To better characterize the patient's holistic pain experience, the Neuropathic Pain Symptom Inventory (NPSI) can also be completed (36). This self-questionnaire will best document the discrimination and quantification of the multiple dimensions frequented in neuropathic pain syndromes and would allow for a sophisticated representation of centralized pain which then better correlates with the patient's pain profile.

Future Direction

During the initial study design, we believed that patients experiencing MS induced neuropathic pain would have an upregulation of MMP-9, MBPCP, or Anti-MBPCP auto-antibodies. To this end, blood samples were collected to quantify any possible studies involving MBPCP or Anti-MBPCP immunoglobulins while urine was collected to quantify any possible MMP levels. Considering that MS is a central demyelinating disorder, biomarkers quantified in the CSF may prove to be another reliable indicator of neuropathic pain. A related set of experiments studying the relationship between immunoglobulin production in the CSF and MS clinical symptom profiles would be interesting to compare against the immunoglobulin production in the blood. This would require amending the current IRB approval to provide consent for a lumbar puncture in order to obtain and study CSF.

Given the preliminary nature of pilot study, we have focused this report on only immunoglobulins as the primary biomarker of study. However other investigators have already studied MBP like peptides in the urine to characterize MBP (35), we will also want to analyze the urine collected in our study to assess whether patients with MS induced neuropathic pain demonstrate a relationship with MMP as well. From our collected urine samples, we plan on utilizing MMP activity assays by using the fluorescent MCA-PLGL-DpA-AR-NH as the MMP cleavage substrate. Additional experiments using gelatin zymography on the collected urine samples will also quantify the overall quantity of MMP. These biochemical experiments will be conducted at laboratories either at UCSD or Sanford Burnham Prebys Discovery Institute and will then be compared against the patient's clinical level of pain.

To our knowledge, there are no other reports studying whether serum auto-antibodies and urine MMP activity correlate with clinical profiles of pain. There is currently a severe lack of quantitative biomarkers of pathologic pain. Consequently the development of bioassays to detect the levels of MMP-9, MBPCP, and Anti-MBPCP antibody may have diagnostic and therapeutic value for multiple pathologies that result in clinical neuropathic pain.

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