I, Gaurav Gulati, hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
Blood Brain Barrier and Anti-NR2 Antibody in SLE Patients with Cognitive Dysfunction

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This work and its defense approved by:

Committee chair: Erin Nicole Haynes, Dr.P.H.
Committee member: Bin Zhang, Ph.D.
Committee member: Michael Luggen, M.D.
Blood Brain Barrier and Anti-NR2 Antibody in SLE Patients With Cognitive Dysfunction

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Master of Science in Clinical & Translational Research

In the Department of Environmental Health Division of Epidemiology & Biostatistics of the College of Medicine

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ABSTRACT

BACKGROUND: Cognitive Dysfunction (CD) is one of the most common manifestations of neuropsychiatric SLE (NPSLE) and one of the most devastating. The pathogenesis of CD in SLE is not known, but in animal models, antibody to the NR2 subunit of the N-methyl D-aspartate receptor (aNR2) can cause memory impairment. However, this effect can only be demonstrated if the blood brain barrier (BBB) has been disrupted or if the antibody is introduced intrathecally. Several studies in SLE patients have failed to find an association of aNR2 with CD. None, however, has assessed the integrity of the BBB as a potential pathogenic cofactor.

S100B protein is an astrocyte specific protein that has been used as biomarker of BBB disruption in traumatic brain injury and some neurodegenerative disorders. And, antibodies to this protein may indicate previous exposure to this immunologically privileged protein and might be used as an indicator of preceding BBB disruption. We hypothesized that aNR2 antibody is pathogenic in SLE patients only if there evidence of previous or ongoing BBB disruption as indicated by increased levels of S100B or anti-S100B.

METHODS: Patients who fulfilled the revised American College of Rheumatology (ACR) criteria for SLE and were stable for at least 4 weeks were recruited from three different settings. Basic demographic, clinical and laboratory data was collected. The Automated Neuropsychological Assessment Metrics (ANAM), a computerized and validated tool, was utilized to measure cognitive function. The Total Throughput Score (TTS = number of correct responses/time) was used as the primary outcome measure. CD was defined as a score of less than 1.5 SD below the age, sex, and race matched RA population mean. Patients also had assessment of fatigue, depression, SLE activity and SLE damage using the FACIT fatigue score, Becks Depression Inventory (BDI), SLEDAI 2K, and SLICC respectively. Serum was analyzed by established ELISA techniques for anti-NR2 antibody, anti-S100B antibody and intact serum S100B protein.
RESULTS: A total of 57 patients were evaluated. Twelve patients had CD. The age, ethnicity, and family income were significantly different between the two groups (p<0.05). In a multiple regression model adjusting for the above independent variables together with simple reaction time and opioid use, no significant effects of aNR2, S100B, or aS100B on decreasing TTS were found. And, when the effects of aNR2 antibodies were evaluated at high levels of S100B and aS100B, no significant influence on TTS was found.

CONCLUSION: Serum antibodies to NR2 do not appear to play a role in the pathogenesis of CD in SLE even when analyzed in the context of BBB integrity. This would suggest that, if these antibodies are pathogenic, they are produced within the CNS and peripheral antibody measurements do not adequately reflect their intrathecal levels.
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CHAPTER 1: INTRODUCTION

Cognitive dysfunction (CD) is one of the most frequent neuropsychiatric manifestations of SLE (1,2). The reported prevalence has varied widely depending on the method of assessment, definition employed, population studied, and controls used. The largest study to date by Murray et al involving 694 subjects estimated the prevalence at of CD in an unselected lupus population at 15% (3). CD is associated with significant work disability, impaired functional capacity, and diminished quality of life through disruption of multiple cognitive domains (4–8).

The pathophysiology of CD in SLE is poorly understood. The N-methyl D-aspartate (NMDA) receptor is a glutamate receptor in the brain and is responsible for synaptic plasticity and memory (9). The NR2 subtype has been found in particularly high density in the hippocampus (10). In the hippocampus, the calcium gated NMDAR is responsible for the neuronal plasticity and Long-Term Potentiation (LTP) required for memory and learning (11). Continuous excitation of the receptor leads to high influx of calcium, with an initial excitatory response followed by neuronal apoptosis (12).

Diamond et al established that some murine monoclonal anti–DNA antibodies cross react with the NR2 receptor and caused neuronal cell death when injected into mouse brains presumably due to excessive excitation (13). This antibody is directed specifically against the “DWEYS” amino acid sequence (Asp-Trp-Glu-Tyr-Ser) which is common to both murine and human NR2 subunits (9,13). The same effects were noted when the human SLE sera containing high titers of anti–ds DNA with cross reactivity to anti-NR2 was injected into mouse brains. However, this antibody was not pathogenic when injected systemically unless the blood brain barrier (BBB) was disrupted (14).
There have been multiple studies on human anti-NR2 levels and its relationship to CD (9). The results have been conflicting (9). Most studies, which have examined correlation of peripheral blood antibody and CD, have been negative. Interestingly, all studies that have examined CSF have reported positive associations. This would suggest that potentially pathogenic anti-NR2 antibodies are either produced intrathecally or else, as in mice, that disruption of the BBB is required for their CNS pathogenicity. Increased permeability of the BBB has been noted in previous studies on NPSLE (9,15) and steroid administration has been shown to lower CSF antibody concentration (16). But, previous studies evaluating the effects of serum anti NR2 levels on cognitive performance have not considered the role of the BBB in their analysis.

The S100B protein is a glial specific protein expressed by endothelial-lining mature astrocytes that help form the BBB (17,18). It is an established biomarker for acute damage to the BBB and has been studied in a variety of chronic neurological diseases (18). Three studies have examined the relationship of S100B and NPSLE in general and have found conflicting results (19–21). There have been no studies that have specifically addressed the relationship of S100B and CD (19–21).

Disruption of the BBB may be transient in response to infections, trauma, drugs, or toxins (22). Transient disruption may be sufficient to permit entrance of pathogenic antibodies into the CNS and yet not be detectable by S100B or other direct measures of BBB integrity performed at a single point in time. To identify intermittent disruption, it would be necessary to longitudinally evaluate these markers, especially at potentially critical times. Alternatively, since S100B is immunologically privileged (18,19), if released into the systemic circulation, it may induce autoantibody formation. Limited work has been done in this area, but anti-S100B antibodies have been found in some chronic neurological diseases like multiple sclerosis and Parkinson’s
disease (23) and as well as in some patients with Alzheimer’s disease (24). Anti-S100B antibodies have also been studied in football players and correlations were found between the number of head “hits” and MRI changes and the titers of the antibody (22). There have not been any studies to date evaluating the role of anti-S100B antibodies and cognitive function in SLE patients.

The purpose of our study therefore was to evaluate the relationship of peripheral blood anti-NR2 antibody with CD in the context of the integrity of the BBB. Our study is the first to attempt to assess this complicated relationship.
CHAPTER 2: METHODS

2.1 Patient Selection

We recruited willing patients from three clinical settings – the University of Cincinnati Medical Center SLE clinic, and the faculty academic and community based private practices. Inclusion criteria for our study were adult SLE patients fulfilling the ACR classification criteria, clinically stable disease activity for at least 4 weeks prior to the study with no medication changes for that period. The University of Cincinnati Institutional Review Board approved the research.

We collected demographic information including age, gender, ethnicity, family income, occupation, and educational attainment. We also ascertained clinical status which included comorbidities, medications, SLE classification criteria met, SLE disease activity index (SLEDAI-2K), SLE chronic damage index (SLICC), and routine lab data by chart review (25,26). Finally, we assessed psychological state with Beck’s Depression Inventory (BDI) and the Functional Assessment of Chronic Illness Therapy Fatigue assessment (FACIT) (27,28). Health Related Quality of Life was also measured with the Short Form 36 (SF-36) (29).

Patients underwent analysis of cognitive function using the Automated Neuropsychologic Assessment Metrics (ANAM), a validated and frequently used tool for cognitive assessment in a number of conditions including in SLE (30,31). The ANAM is a self administered, computer based test that takes about a half an hour to complete, requires minimal training to administer and has been successfully studied in mixed ethnic backgrounds (32). It was designed to assess the cognitive domains similar to traditional neuropsychological testing but does not need a highly trained professional to administer, and is less time intensive. The ANAM4 includes simple reaction time (SRT) and 8 subtests that measure short term and long term recall, learning,
working memory, sustained attention, logical reasoning, and mathematical and visual-spatial processing. The SRT is the time required to depress the mouse button after seeing a symbol flashed onto the computer screen. It measures neuromuscular efficiency (which may be affected by arthritis, tendonitis, neuropathy, etc.) and permits adjustment for this in subsequent analyses. For each subtest, a number of parameters are reported, including accuracy, mean and median response times, standard deviations of response times, and throughput. The throughput is the number of correct responses divided by the time required for the correct responses. The Total Throughput Score (TTS) is the sum of the throughput scores for each of the eight domains. Patients were categorized as having cognitive dysfunction if their TTS was more than 1.5 SD below the mean of an age and sex and race matched rheumatoid arthritis control population on two assessments, a minimum of 6 months apart (30).

We collected serum samples from the patients at the time of their cognitive assessments. These were aliquoted and frozen at -80°C until analyzed as a group.

2.2 Biomarker Assays

Serum anti NR2 antibody levels were measured by ELISA using the 10 amino acid peptide sequence “DWEYSVWLSN” described by DeGiorgio et al previously and the Optical Density (O.D.) values measured were reported (13).

S100B measurements were performed using an ELISA kit (96 wells, anti-human S100B, Diasorin, Stillwater, MN) and reading done using a multi-plate fluorescent reader. Fluorescent signals were converted into ng/ml as per standard curve concentrations.
The measurement of serum S100B autoantibodies were performed based on an ELISA method described in the article by Marchi et al (22). First, 96 well plates were coated with a PBS solution containing S100B protein (human brain, catalog number-559291, EMD Chemicals). Optimization of this ELISA was achieved by testing two concentrations of S100B protein (1 or 5 µg per well). No significant differences were observed at these two concentrations of S100B coating. A coating solution of 1µg per well was used. An S100B monoclonal antibody (catalog number: Q86610M, Meridian Life Science Inc.) was used as a standard to allow for the conversion of absorbance unit (AU) values into concentrations (µg/ml) or titer (number of dilutions). Standard curves were obtained with 100 µl of serially diluted S100B monoclonal antibodies. Plates were coated overnight at 4°C with S100B protein (1 µg/well). Wells were then washed 3 times with PBS. Subsequently, 100 µL of a 1% BSA blocking solution was added in each well and incubated for 2 hours at room temperature. Wells were then washed 3 times with 200 mL of PBS containing 0.05% Tween-20. Serum samples and standards were added and incubated for 1 hour at room temperature. Samples were aspirated and wells washed 3 times using 200 µL of PBS containing 0.05% Tween-20. A secondary antibody solution of 200 mL of horseradish-peroxidase (HRP) goat anti-mouse IgG and 200 mL of HRP goat anti-human were added to the standards and serum samples respectively. After a 1 hour incubation at room temperature wells were washed 3 times with 200 µL of PBS containing 0.05% Tween-20. Finally, 100 µL of OPD solution was added and the reaction incubated for 30 minutes at room temperature. The reaction was stopped by adding 100 µL of 2.5 M Sulfuric acid. Samples were analyzed using an ELISA plate reader at 490 nm.

2.3 Statistical Analysis
Data entry was done on Microsoft Excel® spreadsheets and analyzed using SAS 9.3® software (33). Patient population was characterized using descriptive statistics. The association between CD status and the categorical variable were evaluated by Chi-Square test or Fisher’s exact test and the continuous variables were compared by either Student’s t-test or Mann-Whitney test between patients with and without CD. Initial evaluation of the relationship between TTS and serum anti-NR2 antibody, serum S100B protein levels, and serum anti-S100B levels was done by Spearman correlation. To assess the potential influence of BBB disruption on the correlation of the TTS and anti-NR2 antibody, patients were also divided into tertiles of S100B and anti-S100B and a similar analysis was performed. In order to adjust for the influence of independent variables (like age, ethnicity, education, disease activity, medications, etc.) on TTS, a multiple regression model was utilized with interaction terms for anti-NR2 and S100B and anti-NR2 and anti-S100B. Some of the variables were not normally distributed, logarithmic transformation was utilized to correct for this. Candidate variables were first identified by a forward stepwise procedure. Logistic regression was also performed to identify independent variables influencing the presence of CD.

Preliminary estimates of power with case: control ratio of approximately 1:4 would suggest that 50 subjects total would be required to achieve an 80% power to detect a 1 SD difference (0.07 OD) in anti-NR2 at the $\alpha = .05$ level.
CHAPTER 3: RESULTS

3.1 Patient Characteristics

For our study we enrolled a total of 57 patients with SLE. Table 1 summarizes the patient demographics and clinical parameters including medication use. The mean age of patients was 49.9 years, 93% were women, and a majority were African Americans (56.1%). Education was limited with 36.8% having ≤ 12 years and 45.6% had a family income of less than USD 20,000 a year. Disease activity measured by the SLEDAI-2K was low (mean ± SD: 3.6 ± 3.4) while the mean chronic damage index (SLICC) score was substantial at 2.75 (SD = 2.4). Patient pain described on a 0 to 100 visual analogue scale (VAS) was 40.0, while overall global assessment by the patient on a similar scale was 53.7. Depression on average was mild with a mean score of 16.2 (±12.3) but 35% were affected to a moderate or severe extent. Fifty-four percent were on prednisone, but only about 16% were on doses exceeding 20 mg. The mean prednisone use was 7.1 mg a day (SD 8.4). Approximately 50% were taking other immunosuppressive medications including methotrexate, mycophenolate and azathioprine (Table 1).

Using the above definition of CD we identified twelve patients who were cognitively impaired. Eleven of the twelve were evaluated to 2 occasions at least 6 mos apart and had persistent CD. The remaining patient has yet to be re-evaluated. Comparing those with to those without CD, we found the differences in age, family income, and ethnicity to be statistically significant (p<0.05). All other parameters including disease duration, education, SLICC, FACIT and SLEDAI scores, patients’ global assessment and pain assessment and medication use were not statistically different between the two groups (Table 1).
3.2 Serum Anti NR2 antibody, S100B Protein, Anti S100B Antibody levels

We compared the anti NR2 antibody levels between patient groups with and without CD and did not find a statistical significant difference (p > 0.05). The same was true with regard to the serum S100B protein levels and anti S100B antibody levels between the two groups. The correlations of TTS with anti NR2 antibody levels, serum S100B protein levels, and anti S100B antibody levels were not significant either (Table 2).

Next, we classified subjects into tertiles stratified according to levels of S100B and anti-S100B, and then examined the relationship of the anti NR2 antibody levels with TTS (Figures 1 and 2). Again no statistically significant associations were found (p > 0.05). To adjust for the influences of age, ethnicity, family income, and other potentially important variables on cognitive function, we utilized a multiple regression model in a forward stepwise manner with TTS as the dependent variable. Because S100B and anti-NR2 were not normally distributed, they were log transformed. The other variables in the model conformed to the assumption of normality. The results are shown in Table 3. The only statistically significant independent variables were age, ethnicity, throughput of Simple Reaction Time (SRT), and opioid use. The levels of anti NR2 antibody, S100B protein levels, and anti S100B antibody levels did not significantly affect TTS. Because anti-NR2 antibody may be deleterious only when there is evidence of BBB disruption, we performed the multiple regression using interaction terms (anti-NR2 and S100B levels and anti-NR2 and anti-S100B antibody levels) which evaluated the influence of anti-NR2 at high and low levels of these markers of BBB disruption. Again no consistent and statistically significant effect of anti-NR2 on TTS was found (Table 4). Finally, we performed a logistic regression to model CD (as defined previously). Apart from age, ethnicity and family income, we found no statistically significant associations (Data not shown).
CHAPTER 4: DISCUSSION

There is persuasive animal data linking cross reactive anti DNA/anti-NR2 antibodies and apoptotic neuronal cell death in mice (13). And studies of anti-NR2 antibodies in the CSF demonstrate a consistent relationship between the anti NR2 antibody levels and cognitive function (16,34,35).

However, previous studies looking at serum anti NR2 antibodies have led to conflicting results with most reporting no significant association, although Brunner et al, studying pediatric patients longitudinally, were recently able to find a correlation of changes in anti-NR2 antibody with changes in some measures of cognition (36). These differences in results are perhaps due to differences in biomarker assay techniques, definition of CD and tools used to measure cognitive function, and perhaps, differences in patient populations (9,31,36–42). The other explanation is that the antibody is produced intrathecally and never enters the systemic circulation (9). Or, alternatively, as in the animal models, the antibody could be produced systemically but is pathogenic only if there has been disruption of the BBB. Our study was designed to assess this latter possibility.

We chose to examine the serum levels of S100B, an astrocytic protein normally not found in peripheral blood, as an indicator of current or recent disruption. It has been demonstrated to be a reliable and valid measure of BBB disruption in other acute and chronic neurologic diseases (24). As has been mentioned, the S100B protein has also been used to assess patients with NPSLE in three studies. Schenatto et al examined the role of S100B protein in their study on NPSLE patients and found S100B levels in serum to be double in NPSLE (median 0.164 ng/ml, inter quartile range 0.113 to 0.332) than the non NPSLE patients (0.062 ng/ml, 0.026 - 0.109) or controls (0.088 ng/ml, 0.013 - 0.124) and these differences were statistically significant (p<
0.001). CSF was not evaluated (20). Subsequently, Yang reported on 157 patients, of which 65 had NPSLE, and found significant differences in both their serum and CSF based samples (21). While Fragoso-Loyo, studying the utility of serum S100B levels in CNS involvement in SLE patients, found no differences in their NPSLE group, either in serum or in CSF (19). In this last study no patients were reported to have CD as their NPSLE manifestation. And, in the studies by Yang et al and Schenatto very few patients had CD and these were not analyzed separately (19–21).

Since disruption of the BBB could occur intermittently and/or could have occurred previously and still have permitted significant CNS injury, we measured antibodies against S100B in serum as a potential indicator of previous BBB disruption. As an immunologically privileged protein, S100B might initiate an immune response in susceptible individuals if released in significant concentrations into the peripheral blood. That autoantibody might persist for months, if not longer. There have been a few previous studies which have examined antibody to S100B in other chronic neurological disorders and head trauma (22–24). The study by Poletaev and colleagues looked at patients with Multiple Sclerosis and Parkinson’s (N = 26 and 20 respectively) and found the prevalence of these antibodies to be significantly higher than controls (p < 0.001) (23). Gruden et al studied the anti S100B antibody levels in Alzheimer’s patients (N = 48) and found that the levels were significantly higher in early cases with moderate dementia, the same was not true for early cases with mild dementia or advanced cases (24). Marchi and colleagues have demonstrated that half of the football players they studied with repetitive head trauma had increased levels of the antibody (p<0.05) which correlated with serum S100B levels and MRI changes (22). But, there have been no previous studies evaluation anti-S100B antibody in patients with SLE.
In our study, we found significant association of the TTS, a measure of cognitive function, with age, ethnicity, Simple Reaction Time (SRT) throughput, and opioid use as discussed above. We were, however, unable to find a significant relationship between the anti-NR2 antibody and CD despite using biomarkers of both recent and remote BBB disruption. We selected our study population from three different sources to make it more representative of the general SLE population and to try to minimize selection bias. We used a validated and reproducible test of cognitive performance, the ANAM, for our study.

We used an age, sex, and race matched control population of RA patients in order to define CD. In doing so, we were able to control for the potential influence of a chronic, debilitating, and painful condition on cognition. Most studies have not used disease controls and may have misclassified subjects as cognitively impaired as a consequence of SLE when, in fact, their dysfunction was due to other non-immunologic factors such as fatigue, anxiety, fear, pain, depression, etc. We also required persistence of CD for at least 6 months in order for a subject to be so classified. This was done to identify the subset of patients of greatest interest—those with persistent or progressive disease and to exclude those with self-limited dysfunction who may not need specific intervention.

We also collected detailed information on potential covariates that might impact cognitive performance including education, income, disease activity and severity, medication use, pain, fatigue, and depression. We adjusted for these potential confounders in our multivariate analyses.

Our study has limitations. We examined a relatively small patient population, especially of those who were cognitively impaired. Power calculations would suggest that this isn’t a major problem. We utilized a cross sectional design for efficiency. However, CD may be a slowly progressive or
an intermittently progressive phenomenon. Or, it may have occurred years previously and left no trace of the responsible pathogenic influence. We would not be able to detect these time-dependent processes reliably, only a prospective study would be able to do so.

In addition, the biomarkers we used to identify BBB disruption may not sufficiently sensitive or specific for this process. No reliable information currently exists comparing these biomarkers to others that might be employed in this context. Our choice, therefore, was somewhat arbitrary. Better biomarkers may exist.

The other explanation for the lack of association of peripheral anti-NR2 antibody and CD is that it is only the intrathecally produced anti-NR2 antibody that is pathogenic and peripheral antibody is irrelevant. However, if the intrathecal anti-NR2 antibody does produce neuronal cell damage and death, this may lead to disruption of the BBB and leakage of the antibody into the peripheral circulation enabling it’s detection, under certain circumstances, to serve as a less invasive diagnostic test for CD.

Further work is needed to understand cognitive dysfunction in SLE. A larger population of SLE patients needs to be studied in order to account for the multiplicity of variables that could potentially influence cognitive function. A prospective study design with multiple sampling times and multiple biomarkers measured longitudinally will be necessary to help unravel the web of cause and effect. Moreover, studies should also assess the possibility that CD is the result of a multi-step process with potentially different pathogenic factors operating at each of the steps.


APPENDICES: TABLES AND FIGURES

TABLE 1: Patient Demographics and Biomarkers Values: Dichotomized By CD

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Subjects (n = 57)</th>
<th>CD (n = 12)</th>
<th>No – CD (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years (SD))</td>
<td>49.9 (11.2)</td>
<td>54.9 (8.8)</td>
<td>48.5 (11.5)*</td>
</tr>
<tr>
<td>Caucasians (%)</td>
<td>36.8</td>
<td>8.3</td>
<td>44.4*</td>
</tr>
<tr>
<td>Family Income &lt; USD 20k (%)</td>
<td>45.6</td>
<td>75.0</td>
<td>37.8*</td>
</tr>
<tr>
<td>Females (%)</td>
<td>92.9</td>
<td>83.3</td>
<td>95.6</td>
</tr>
<tr>
<td>Education &lt; 12 years (%)</td>
<td>36.8</td>
<td>50.0</td>
<td>33.3</td>
</tr>
<tr>
<td>SLE Disease Duration (Years)</td>
<td>13.1 (10.1)</td>
<td>17.5 (13.9)</td>
<td>12.0 (8.6)</td>
</tr>
<tr>
<td>SLEDAI (Mean (SD))</td>
<td>3.6 (3.4)</td>
<td>3.2 (4.3)</td>
<td>3.8 (3.3)</td>
</tr>
<tr>
<td>SLICC (Mean (SD))</td>
<td>2.75 (2.4)</td>
<td>3.4 (2.1)</td>
<td>2.4 (2.4)</td>
</tr>
<tr>
<td>Pain (100 mm VAS - Patient)</td>
<td>40.0 (28.2)</td>
<td>49.6 (29.1)</td>
<td>36.2 (27.7)</td>
</tr>
<tr>
<td>Global Assessment (100 mm VAS - Patient)</td>
<td>53.7 (22.6)</td>
<td>58.8 (22.6)</td>
<td>52.3 (22.7)</td>
</tr>
<tr>
<td>BDI II (Depression) (Mean (SD))</td>
<td>16.0 (12.3)</td>
<td>17.6 (10.2)</td>
<td>14.4 (12.6)</td>
</tr>
<tr>
<td>FACIT (Fatigue) (Mean (SD))</td>
<td>24.6 (13.5)</td>
<td>26.1(10.8)</td>
<td>23.3 (14.3)</td>
</tr>
<tr>
<td>APL Positive (%)</td>
<td>38.6</td>
<td>25.0</td>
<td>42.2</td>
</tr>
<tr>
<td>Current Prednisone Use (%)</td>
<td>53.7</td>
<td>54.5</td>
<td>53.9</td>
</tr>
<tr>
<td>Prednisone &gt; 20 mg/d (%)</td>
<td>15.8</td>
<td>25.0</td>
<td>13.3</td>
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<tr>
<td>Immunosuppressant Use (%)</td>
<td>48.2</td>
<td>36.4</td>
<td>51.2</td>
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<tr>
<td>Plaquenil Use (%)</td>
<td>70.0</td>
<td>50.5</td>
<td>74.4</td>
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<tr>
<td></td>
<td>CD 1</td>
<td>CD 2</td>
<td>CD 3</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Warfarin (%)</td>
<td>14.8</td>
<td>18.2</td>
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<td>Aspirin (%)</td>
<td>38.9</td>
<td>27.3</td>
<td>41.9</td>
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<td>Antidepressants (%)</td>
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<td>Opioid (%)</td>
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<td>45.5</td>
<td>20.9</td>
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<tr>
<td>NSAID (%)</td>
<td>22.2</td>
<td>18.2</td>
<td>23.3</td>
</tr>
<tr>
<td>Serum anti-NR2 Antibody (SD)</td>
<td>0.41 (0.24)</td>
<td>0.45 (0.19)</td>
<td>0.40 (0.26)</td>
</tr>
<tr>
<td>Serum anti-S100B Antibody (SD)</td>
<td>0.57 (0.11)</td>
<td>0.58 (0.09)</td>
<td>0.57 (0.11)</td>
</tr>
<tr>
<td>Serum S100B Protein (SD)</td>
<td>0.09 (0.05)</td>
<td>0.09 (0.05)</td>
<td>0.09 (0.05)</td>
</tr>
</tbody>
</table>

* Indicates a significant difference between the CD and no-CD groups (p <0.05)
TABLE 2: Correlation Analysis Between Biomarkers and TTS*

<table>
<thead>
<tr>
<th>Comparison Groups</th>
<th>Correlation Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTS versus Anti-NR2</td>
<td>-0.059</td>
<td>0.6616</td>
</tr>
<tr>
<td>TTS versus S100B</td>
<td>-0.161</td>
<td>0.2302</td>
</tr>
<tr>
<td>TTS versus Anti S100B</td>
<td>-0.011</td>
<td>0.9334</td>
</tr>
<tr>
<td>S100B versus Anti S100B</td>
<td>-0.132</td>
<td>0.3276</td>
</tr>
</tbody>
</table>

*Spearman’s Correlation Coefficient
### TABLE 3: MULTIPLE REGRESSION ANALYSIS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>SE</th>
<th>t Value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>341.34</td>
<td>120.17</td>
<td>2.84</td>
<td>0.007</td>
</tr>
<tr>
<td>Log (antiNR2)</td>
<td>71.54</td>
<td>70.23</td>
<td>1.02</td>
<td>0.314</td>
</tr>
<tr>
<td>Log (S100b)</td>
<td>22.91</td>
<td>54.40</td>
<td>0.42</td>
<td>0.676</td>
</tr>
<tr>
<td>Anti-S100b</td>
<td>56.45</td>
<td>150.70</td>
<td>0.37</td>
<td>0.710</td>
</tr>
<tr>
<td>Log (AntiNR2) * Log (S100B)</td>
<td>-40.71</td>
<td>65.66</td>
<td>-0.62</td>
<td>0.639</td>
</tr>
<tr>
<td>Log (Anti NR2) * Anti-S100B</td>
<td>4.38</td>
<td>73.21</td>
<td>.06</td>
<td>0.953</td>
</tr>
<tr>
<td>Log (S100B)*Anti-S100B</td>
<td>-49.82</td>
<td>73.21</td>
<td>-1.59</td>
<td>0.119</td>
</tr>
<tr>
<td>Simple Reaction Time (Throughput)</td>
<td>0.600</td>
<td>0.17</td>
<td>3.43</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (per year increase)</td>
<td>-0.008</td>
<td>0.002</td>
<td>-3.71</td>
<td>0.001</td>
</tr>
<tr>
<td>Opioid Use</td>
<td>-82.93</td>
<td>22.05</td>
<td>-3.76</td>
<td>0.001</td>
</tr>
<tr>
<td>Caucasian Ethnicity</td>
<td>76.60</td>
<td>22.27</td>
<td>3.44</td>
<td>0.001</td>
</tr>
<tr>
<td>Income Level (Low)</td>
<td>-15.89</td>
<td>23.86</td>
<td>-0.67</td>
<td>0.509</td>
</tr>
</tbody>
</table>

*a Dependent Variable: Total Throughput Score (TTS); Method Used: forward stepwise multiple regression

* Interaction between two variables such that the effect of one depends on the level of the other
FIGURE 1: Tertiles Of S100B Protein Levels: anti NR2 Antibody versus TTS

A., B. and C. Represent First, Second and Third Tertiles of Serum S100B, Respectively.
FIGURE 2: Tertiles Of anti-S100B Ab Levels: anti NR2 Antibody versus TTS

A., B. and C. Represent First, Second and Third Tertiles of Serum anti-S100B Ab, Respectively.