Evaluation of the Cunningham Panel™ in pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS) and pediatric acute-onset neuropsychiatric syndrome (PANS): Changes in antineuronal antibody titers parallel changes in patient symptoms


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ABSTRACT

Objective: This retrospective study examined whether changes in patient pre- and post-treatment symptoms correlated with changes in anti-neuronal autoantibody titers and the neuronal cell stimulation assay in the Cunningham Panel in patients with Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infection (PANDAS), and Pediatric Acute-onset Neuropsychiatric Syndrome (PANS).

Methods: In an analysis of all tests consecutively performed in Moleculera Labs' clinical laboratory from April 22, 2013 to December 31, 2016, we identified 206 patients who were prescribed at least one panel prior to and following treatment, and who met the PANDAS/PANS diagnostic criteria. Patient follow-up was performed to collect symptoms and treatment or medical intervention. Of the 206 patients, 58 met the inclusion criteria of providing informed consent/assent and documented pre- and post-treatment symptoms. Clinician and parent-reported symptoms after treatment or medical intervention were categorized as "Improved/Resolved" (n = 34) or "Not-Improved/Worsened" (n = 24). These were analyzed for any association between changes in clinical status and changes in Cunningham panel test results. Clinical assay performance was also evaluated for reproducibility and reliability.

Results: Comparison of pre- and post-treatment status revealed that the Cunningham Panel results correlated with changes in patient's neuropsychiatric symptoms. Based upon the change in the number of positive tests, the overall accuracy was 86%, the sensitivity and specificity were 88% and 83% respectively, and the Area Under the Curve (AUC) was 93.4%. When evaluated by changes in autoantibody levels, we observed an overall accuracy of 90%, a sensitivity of 88%, a specificity of 92% and an AUC of 95.7%. Assay reproducibility for the calcium/calmodulin-dependent protein kinase II (CaMKII) revealed a correlation coefficient of 0.90.

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(p < 1.67 × 10^{-6}) and the ELISA assays demonstrated test-retest reproducibility comparable with other ELISA assays.

Conclusion: This study revealed a strong positive association between changes in neuropsychiatric symptoms and changes in the level of anti-neuronal antibodies and antibody-mediated CaMKII human neuronal cell activation. These results suggest there may be clinical utility in monitoring autoantibody levels and stimulatory activity against these five neuronal antigen targets as an aid in the diagnosis and treatment of infection-triggered autoimmune neuropsychiatric disorders. Future prospective studies should examine the feasibility of predicting antimicrobial and immunotherapy responses with the Cunningham Panel.

1. Introduction

The biology behind many neuropsychiatric conditions remains elusive, but recent studies implicate immune dysregulation in some cases, particularly the presence of autoantibodies targeting neural tissue. Numerous studies have linked movement, behavior, and neuropsychiatric disorders to infections and the production of anti-neuronal autoantibodies (Kirvan et al., 2003; Cox et al., 2013; Kirvan et al., 2006a, 2006b; Kirvan et al., 2007; Singer et al., 2015; Brimberg et al., 2012; Cunningham, 2012, 2014; Garvey et al., 1999; Greenberg, 2017; Murphy et al., 2007; Perlmutter et al., 1998; Taranta and Stollerman, 1956; Taranta, 1959; Swedo, 1994; Cox et al., 2015; Rhee and Cameron, 2012). Infectious triggers such as streptococcal and other infections, along with anti-neuronal autoantibodies similar to those associated with Sydenham chorea have been linked to childhood obsessive-compulsive disorder (OCD) and/or tics (Kirvan et al., 2003; Kirvan et al., 2006a, 2006b; Swedo, 1994; Swedo et al., 1998; Swedo et al., 1997; Swedo et al., 1993; Swedo et al., 1989). When symptom onset is abrupt, Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infection (PANDAS) and Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) are two disorders used to describe these symptoms. The diagnostic criteria for PANS is defined as an abrupt onset of OCD or severely restricted food intake, and the presence of at least two of the following seven categories: (1) anxiety; (2) emotional lability and/or depression; (3) irritability, aggression, and/or severely oppositional behaviors; (4) behavioral (developmental) regression; (5) deterioration in school performance (related to attention deficit hyperactivity disorder-like symptoms, memory deficits, cognitive changes); (6) sensory or motor abnormalities; (7) somatic signs and symptoms, including sleep disturbances, enuresis, or increased urinary frequency (Swedo et al., 1997; Chang et al., 2015).

Since Sydenham chorea has a well-established biological mechanism connecting streptococcal infections with autoimmune-induced neuropsychiatric symptoms, it has been used as a biological model to better understand PANDAS and PANS (Cunningham, 2012, 2014). Central nervous system (CNS) autoimmune targets that were originally identified through multiple studies in patients with Sydenham chorea were applied to patients with PANDAS. Targets identified to overlap between Sydenham chorea and PANDAS were used to develop the Cunningham Panel, a set of blood tests utilized for measuring immune dysfunctions, related to neuropsychiatric conditions associated with an infectious trigger.

1.1. Biological basis of assays in the Cunningham Panel

The Cunningham Panel includes five assays performed on a serum sample from blood collected in glass tubes free of any excipients. Four assays measure human serum Immunoglobulin G (IgG) levels by Enzyme-Linked ImmunoSorbent Assays (ELISA) directed against 1) Dopamine D1 Receptor (D1R), 2) Dopamine D2L Receptor (D2LR), 3) Lysoganglioside-GM1 and 4) Tubulin. A fifth assay is a cell stimulation assay which measures the ability of a patient’s serum immunoglobulin G (IgG) to stimulate calcium/calmodulin-dependent protein kinase II (CaMKII) activity in human neuronal cells. We briefly review the biological basis for the selection of these five assays below. (See Fig. 1)

1.1.1. Anti-Lysoganglioside GM1 assay

Commercially available measurements of human group A streptococcal (GAS) antibodies (i.e., ASO and/or anti-DNase B antibodies) are sufficient for measuring GAS reactivity, but do not provide any relationship to GAS-related autoimmune reactivity and the CNS. Using human monoclonal antibodies derived from Sydenham chorea patients, Cunningham and Kirvan observed significant cross-reactivity against neurons in the human basal ganglia (Kirvan et al., 2003) and against N-acetyl-beta D-glucosamine (GlcNAc), the major constituent of the GAS cell wall, a carbohydrate epitope. They observed strong cross-reactivity against the neuronal surface antigen lysoganglioside GM1 but not against other gangliosides (Kirvan et al., 2003; Kirvan et al., 2007). Lysoganglioside GM1 antigen also blocked binding of these autoantibodies to human caudate and putamen, demonstrating specific neural targets in the brain center known to be involved in movement disorders such as chorea, tics and motor stereotypies, whereas these serum autoantibodies receded to normal levels during convalescence. In addition, IgG derived from the serum or cerebrospinal fluid (CSF) of Sydenham chorea and PANDAS patients was found to target human caudate and putamen brain tissue, and this reaction could be inhibited by lysoganglioside GM1 (Kirvan 2006; Kirvan et al., 2006a, 2006b).

![Fig. 1. Autoantibodies directed against Dopamine D1/D2 Receptors and Lysoganglioside GM1, and CaMKII activation. Reprinted by permission from Springer Nature: Nature Reviews Disease Primers, Carapetis et al. Volume 2, "Acute Rheumatic Fever and Rheumatic Heart Disease." 2016.](image-url)
1.1.2. CaMKII cell stimulation assay

Integrated into the Cunningham Panel is the measurement of CaMKII activation which is mechanistically important since activation increases the activity of tyrosine hydrolase, an enzyme that produces dopamine, resulting in increased dopamine output, a key neurotransmitter involved in movement disorders. Both IgG from sera and CSF of PANDAS patients were found to signal activation of CaMKII in human neuronal cells (Kirvan et al., 2006a, 2006b). Supporting the specificity of GAS antibodies causing CaMKII activation, it was demonstrated that activation was blocked by streptococcal-associated GlcNAc or by depleting immunoglobulins from serum and CSF by affinity column adsorption (Kirvan et al., 2006a, 2006b). Additional data showed that CaMKII was activated 202% above basal level in Sydenham chorea and PANDAS patients during the acute phase, whereas convalescent serum collected in the absence of chorea showed no significant increase in CaMKII activity (Kirvan 2003). Finally, additional studies have shown that patient symptom improvement was associated with a reduction in CaMKII activation.

CaMKII is also involved in the regulation of N-methyl-D-aspartate (NMDA) receptor excitability via glutamate transmission (Hell, 2014) which is being recognized in syndromes which include OCD, tics, and Tourette Syndrome (Marsili et al., 2017), and being recognized as a treatment target in OCD (Laoutidis et al., 2016). Mutations in CAMK2A and CAMK2B, the genes that code for CaMKII, have been associated with intellectual disability (Kury et al., 2017) and ASD-related behaviors such as hyperactivity, social interaction deficits, and repetitive behaviors (Stephenson et al., 2017). Other studies have linked CaMKII to the pathogenesis and symptoms in a variety of mental and neurological illnesses, including learning disorders, cognitive impairment, schizophrenia (Robison, 2014; Kury et al., 2017), ischemia, Alzheimer’s disease (Li and Song, 2011; Ghosh and Giese, 2015), epilepsy (Zhang et al., 2014; Robison, 2014) and Parkinson’s disease (Zhang et al., 2014; Zaichick et al., 2017).

1.1.3. Anti-dopamine D1 and D2L receptor

The inclusion of dopamine D1 and D2L receptors as targets arose from studies that demonstrated autoantibodies directed against dopamine D1 and D2L receptors correlated with various neuropsychiatric symptoms (Ben-Pazi et al., 2013). Two types of anti-neuronal human antibodies against the dopamine receptors induced an increase in dopamine neurotransmitter release (Kirvan et al., 2006a, 2006b). D1 and D2L receptor antibodies were elevated in patients with Sydenham chorea and PANDAS compared to controls (Kirvan et al., 2003; Kirvan et al., 2006a, 2006b; Kirvan et al., 2007; Cox et al., 2013; Cox et al., 2015; Cunningham and Cox, 2016), and are likely important in pathogenesis of neuropsychiatric diseases associated with infection (Singer et al., 2015; Brimberg et al., 2012).

1.1.4. Anti-tubulin

Human monoclonal antibodies derived from patients with Sydenham chorea, reacted with human caudate and putamen brain sections and reactivity was blocked by anti-tubulin monoclonal antibodies. The reactive epitope of these brain proteins was found to be a N-terminal amino acid sequence with corresponding homology to β-tubulin (Kirvan et al., 2007). Tubulin autoantibodies have also been identified in chronic inflammatory demyelinating polyneuropathy and Guillain-Barré syndrome (Connolly and Pestronk, 1997), Graves’ disease and Hashimoto’s thyroiditis (Rouset et al., 1983).

1.2. Purpose of this study

The purpose of our study was to determine if, and to what extent, the assays of the Cunningham Panel parallel changes in PANDAS/PANS symptoms with treatment. Since immunomodulatory treatment for neuropsychiatric conditions can have positive (Perlmuter et al., 1999; Kovacevic et al., 2015) but also variable (Williams et al., 2016) outcomes, the ability to predict responsiveness to immunomodulatory treatments would be of high value. As a step towards this goal, this study examines whether the assays within the Cunningham Panel are potential biological markers that can be used to follow changes in symptoms with treatment, and could potentially be used to predict response to immunomodulatory treatment.

2. Methods

2.1. Subjects

All patients presented with various neuropsychiatric symptoms characteristic of the criteria for PANS/PANDAS and were either diagnosed with, or suspected of, PANS/PANDAS at the time of their first test requisition (Table 1). Fifty eight of 206 subjects who were identified to have had a Cunningham Panel from April 22, 2013 through Dec. 31, 2016, had two or more panels performed and met the inclusion criteria (see Fig. 2). Documentation of patient symptoms was received by direct

Table 1

Summary of symptoms of PANS/PANDAS patients included in this study by individual patients in Group 1: improved/resolved and Group 2: not improved/worsened.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Group 1 Improved/resolved</th>
<th>Group 2 Not Improved/worsened</th>
<th>Combined All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count (N = 34)</td>
<td>Percent</td>
<td>Count (N = 24)</td>
</tr>
<tr>
<td>Decreased concentration</td>
<td>31</td>
<td>91%</td>
<td>22</td>
</tr>
<tr>
<td>OCD</td>
<td>34</td>
<td>100%</td>
<td>18</td>
</tr>
<tr>
<td>Emotional lability or depression</td>
<td>30</td>
<td>88%</td>
<td>19</td>
</tr>
<tr>
<td>Sensory symptoms</td>
<td>26</td>
<td>77%</td>
<td>22</td>
</tr>
<tr>
<td>Anxiety: general and/or Separation</td>
<td>26</td>
<td>77%</td>
<td>22</td>
</tr>
<tr>
<td>Sleep disorders</td>
<td>29</td>
<td>85%</td>
<td>15</td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>27</td>
<td>79%</td>
<td>17</td>
</tr>
<tr>
<td>Tics</td>
<td>22</td>
<td>65%</td>
<td>21</td>
</tr>
<tr>
<td>Motor symptoms</td>
<td>19</td>
<td>56%</td>
<td>23</td>
</tr>
<tr>
<td>Developmental regression</td>
<td>23</td>
<td>68%</td>
<td>19</td>
</tr>
<tr>
<td>Dysgraphia</td>
<td>22</td>
<td>65%</td>
<td>18</td>
</tr>
<tr>
<td>Urinary urgency or frequency</td>
<td>15</td>
<td>44%</td>
<td>11</td>
</tr>
<tr>
<td>Chorea/choreiform movements</td>
<td>12</td>
<td>35%</td>
<td>13</td>
</tr>
<tr>
<td>Behavioral regression</td>
<td>8</td>
<td>24%</td>
<td>1</td>
</tr>
<tr>
<td>Anorexia or ARFID</td>
<td>3</td>
<td>9%</td>
<td>3</td>
</tr>
<tr>
<td>Psychosis</td>
<td>4</td>
<td>12%</td>
<td>1</td>
</tr>
</tbody>
</table>

OCD = obsessive compulsive symptoms; ARFID = avoidant/restrictive food intake disorder.

C. Shimazaki, et al.

Journal of Neurommunology 339 (2020) 577138
phone and/or email communication with the prescribing clinician or parents, supported by documented call notes in our Laboratory Information Management System (LIMS). Clinician or parent assessments were specific to improvement or non-improvement compared to their initial presentation of symptoms when the first panel was performed. Based upon post-treatment assessments, patients were categorized into one of two groups: Group 1: “Improved/Resolved” (n = 34) or Group 2: “Not-Improved/Worsened” (n = 24) compared to pre-treatment symptoms. Informed consent/assent was reviewed and approved by the Western Institutional Review Board (WIRB). Since this was a retrospective analysis, parties making assessment were not necessarily blinded to Cunningham Panel results. All patients received medical attention, and 56 of the 58 patients received medications including therapy for infections and/or autoimmune disorders as potential causative factor(s) and/or psychotropic drugs for patient’s neuropsychiatric symptoms. Of the two patients who did not receive medications between testing, one patient could not afford IVIg and therefore did not receive medication, and the other patient’s medical treatment was watchful waiting or a “tincture of time.”

2.2. Cunningham Panel assays

Whole blood or serum samples were received according to standard operating procedures, collected in red-top glass tubes without additives (Covidien, Monoject Red Stopper Blood Collection Tube, glass 7 mL draw, part #8881301512). Whole blood was spun to collect serum. Patient serum was tested according to standard operating procedures for Moleculera Labs’ CLIA/COLA accredited laboratory (CLIA Number 37D2082408). Assay protocols have been described previously (Cox et al., 2013; Kirvan et al., 2003; Brimberg et al., 2012; Kirvan et al., 2006a, 2006b). The threshold for a positive response for the four ELISA assays was set at the mean value of normal controls plus two times the standard deviation, followed by rounding to the nearest titer. In each case, the selected threshold exceeded the 95% confidence interval for the t-distribution. A positive titer result for DR1 is 4000 or higher, for DR2 is 16,000 or higher, for lysoganglioside GM1 is 640 or higher, and for tubulin is 2000 or higher. A positive result for CaMKII activation in SK-N-SH cells was set at 130, or ≥30% above basal control sample. Initially, a population of 20 pediatric controls utilizing designated inclusion/exclusion criteria was obtained from the National Institute of Mental Health, Bethesda, MD (Courtesy of Dr. Susan Swedo) and the Yale Child Study Center, New Haven, CT (courtesy of Dr. James Leckman and Dr. Ivana Kawikova) (Singer et al., 2015). Since then, additional populations of pediatric controls have been tested with similar results.

2.3. Data and methods of analysis

Two distinct approaches were used to evaluate the results of these five assays. The first method (Positive Test Count Method) counted the change in the number of positive assays. The second method (Magnitude of Change Method) examined the quantitative change in each of the five assays using multivariate logistic regression analysis. The latter method examines the magnitude of change and is independent of the cutoff assigned by controls. Multivariate logistic regression analysis is effective when multiple test values may be better predictors compared to any single measure, and is used in other areas of medicine for predicting treatment outcome (Sparano et al., 2018; Hambardzumyan et al., 2015; Van Den Eeden et al., 2018).

2.3.1. Positive test count score

The Positive Test Count Score is the difference in the number of
positive tests pre-treatment, versus the number of positive tests post-treatment. An increase in the number of positive tests results in a negative Positive Test Count Score, an unchanged number results in a score of 0, and a decrease in the number of positive tests results in a positive score. We represent this by the following equation: Positive Test Count Score = \( X_{tub,pre} + X_{D1R,pre} + X_{D2R,pre} + X_{lyso,pre} + X_{CaMKII,pre} \) - \( X_{tub,post} + X_{D1R,post} + X_{D2R,post} + X_{lyso,post} + X_{CaMKII,post} \), where \( X = 1 \) for a positive test and \( 0 \) for a negative test, the subscripts "tub," "D1R," "D2R," "lyso" and "CaMKII" indicate the assay and the subscripts "pre" and "post" indicate whether the assay was conducted before or after treatment, respectively.

### 2.3.2. Magnitude of change score

The Magnitude of Change Score examines the magnitude of change in post-treatment test results compared to pre-treatment. This is defined by the equation: Magnitude of Change Score = \( a_0 + a_1 \times DV_{tub} + a_2 \times DV_{D1R} + a_3 \times DV_{D2R} + a_4 \times DV_{lyso} + a_5 \times DV_{CaMKII} \) where \( DV = \log_2 (Titer_{post}/Titer_{pre}) \) for each of the four ELISA assays and \( DV_{CaMKII} = CaMKII_{value,post} - CaMKII_{value,pre} \). The resulting logistic regression model is then cross-validated using the "leave one out" method. The probability of membership in Group 1, the group with symptomatic improvement, is calculated from the individual's Magnitude of Change Score: \( P = e^{Magnitude Score}/(e^{Magnitude Score} + 1) \). The resulting logistic regression model is then cross-validated using the "leave one out" method. Note that Magnitude of Change Score is independent of the thresholds used to determine individual assay results as positive or negative; instead, Magnitude of Change Score is a function combining the direction and magnitude of changes in all individual tests in the Cunningham Panel. We investigated other models in which selected assays in the Cunningham model were omitted; our findings were essentially unchanged from the full model.

### 2.4. Reproducibility of the Cunningham Panel

Extensive reproducibility testing was conducted using patient blood samples collected in validated glass tubes without excipients (Red Top glass tubes), and tested repetitively at random intervals over a period of 33 months. The reproducibility testing for D1R is representative of each of these tests. Note that a two-fold dilution scheme is used to determine titers. To assess robustness and ongoing assay reproducibility of the
Table 3B
Patients with no improvement in or worsening of symptoms (Group 2, N = 24).

<table>
<thead>
<tr>
<th>Case #</th>
<th>Pretreatment</th>
<th>Post treatment</th>
<th>Number Elevated Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1R D2R Tubulin lyso- ganglioside Gm1 CaMKII</td>
<td>D1R D2R Tubulin lyso- ganglioside Gm1 CaMKII</td>
<td>Pretreatment Post Treatment</td>
</tr>
<tr>
<td>24</td>
<td>1000 8000 500 80 119</td>
<td>2000 8000 250 320 105</td>
<td>0 0</td>
</tr>
<tr>
<td>1</td>
<td>1000 8000 500 80 119</td>
<td>2000 8000 250 320 105</td>
<td>0 0</td>
</tr>
<tr>
<td>14</td>
<td>1000 250 25 25 121</td>
<td>2000 200 400 80 116</td>
<td>0 0</td>
</tr>
<tr>
<td>16</td>
<td>1000 250 25 160 115</td>
<td>2000 200 100 20 148</td>
<td>0 0</td>
</tr>
<tr>
<td>34</td>
<td>2000 200 500 320 121</td>
<td>1000 200 200 40 141</td>
<td>0 0</td>
</tr>
<tr>
<td>25</td>
<td>1000 200 500 80 138</td>
<td>1000 200 250 160 127</td>
<td>1 0</td>
</tr>
<tr>
<td>62</td>
<td>2000 4000 1000 80 216</td>
<td>2000 200 500 80 123</td>
<td>1 0</td>
</tr>
<tr>
<td>64</td>
<td>500 1000 250 320 134</td>
<td>1000 4000 2000 80 125</td>
<td>1 0</td>
</tr>
<tr>
<td>104</td>
<td>2000 4000 1000 40 149</td>
<td>4000 4000 200 80 158</td>
<td>1 0</td>
</tr>
<tr>
<td>41</td>
<td>2000 4000 500 80 217</td>
<td>4000 16000 1000 320 139</td>
<td>1 0</td>
</tr>
<tr>
<td>4</td>
<td>1000 8000 1000 40 219</td>
<td>8000 8000 4000 128 140</td>
<td>1 0</td>
</tr>
<tr>
<td>16</td>
<td>1000 1000 1000 160 179</td>
<td>8000 8000 4000 128 140</td>
<td>1 0</td>
</tr>
<tr>
<td>48</td>
<td>1000 2000 2000 80 156</td>
<td>2000 8000 1600 100 145</td>
<td>1 0</td>
</tr>
<tr>
<td>36</td>
<td>2000 1600 1000 160 164</td>
<td>8000 8000 4000 160 123</td>
<td>2 2</td>
</tr>
<tr>
<td>122</td>
<td>500 8000 1000 40 160</td>
<td>2000 4000 4000 320 145</td>
<td>2 2</td>
</tr>
<tr>
<td>42</td>
<td>12000 4000 2000 80 112</td>
<td>4000 16000 2000 160 164</td>
<td>2 2</td>
</tr>
<tr>
<td>55</td>
<td>2000 8000 1000 320 160</td>
<td>4000 16000 8000 80 152</td>
<td>2 4</td>
</tr>
<tr>
<td>41</td>
<td>8000 250 1000 160 159</td>
<td>4000 16000 4000 80 152</td>
<td>4 4</td>
</tr>
<tr>
<td>26</td>
<td>4000 12000 8000 320 94</td>
<td>8000 16000 8000 1280 177</td>
<td>4 3</td>
</tr>
<tr>
<td>14</td>
<td>8000 4000 1000 640 148</td>
<td>8000 16000 1600 40 92</td>
<td>4 3</td>
</tr>
<tr>
<td>51</td>
<td>8000 12000 8000 320 142</td>
<td>12000 12000 6000 940 113</td>
<td>4 3</td>
</tr>
<tr>
<td>35</td>
<td>4000 12000 4000 160 130</td>
<td>8000 12000 4000 1280 149</td>
<td>4 4</td>
</tr>
</tbody>
</table>

Heat map of the results of the Cunningham Panel. Values for patients with no improvement in or worsening of symptoms after treatment (Group 2, N = 24). The results and the number of elevated tests before and after treatment are shown for each patient in this study. Elevated test values are highlighted in red; the intensity of color is indicative of magnitude of elevation above threshold. The count of elevated individual tests within each patient’s panels before and after treatment are indicated by blue and orange horizontal bars, respectively.

Table 4
Patient numbers by group: impact of treatment on Cunningham Panel results, Comparison of the number of patients showing improvement in symptoms with those showing a decrease in the number of individual positive assays in the Cunningham Panel by populations described in Table 3A and 3B. The p-value was calculated using the Fisher exact test.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Number Positive Tests Post-Treatment vs. Pretreatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No Positive Tests (Pre- or Post-treatment)</td>
<td>Decreased Count (at least 1 positive test)</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>22 (62%)</td>
<td>9 (26%)</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>2 (8%)</td>
<td>1 (8%)</td>
</tr>
</tbody>
</table>

CaMKII assay, routine repeat testing across multiple samples was performed.

3. Results

3.1. Patient demographics

Patients were either diagnosed with, or suspected of, PANS/PANDAS at the time of their test requisition. All 58 patients presented with various neuropsychiatric symptoms characteristic of the criteria for PANS/PANDAS (Table 1). Age and gender distribution for all 58 patients at the time of first testing ranged from 2 to 23 years, whereas the mean and median age for patients was 12.2 ± 4.5 and 12.0 years, respectively. The mean time between the first and second Cunningham Panels in Group 1 was 68.1 weeks, versus 66.2 weeks in Group 2 (p = 0.87). The median time between tests for Group 1 was 48 weeks, versus 62 weeks for Group 2. An interesting observation in examining symptom frequency is that patients who improved with therapy (Group 1) had a higher percentage of OCD, behavioral regression and sleep disorders, whereas those that did not improve with therapy (Group 2) had a higher percentage of tic and movement disorders.

3.1.1. Subjects in group 1: improved/resolved in symptoms (N = 34)

The group of patients reporting symptoms Improved/Resolved post-treatment consisted of 34 individuals ranging in age from 5 to 21 years with a mean age of 12.2 years. There were 13 females (38%) and 21 males (62%). See Table 2.

3.1.2. Subjects in group 2: not improved/worsened in symptoms (N = 24)

The group of patients reporting symptoms Not Improved/Worsened consisted of 24 patients ranging in age from 2 years to 23 years with a mean age of 12.1 years. There were 9 females (38%) and 15 males (62%), and there were no statistically significant differences in age or gender between Group 1 and Group 2. There were no statistically significant differences in the time between the first test and the second test in Group 1 versus Group 2 (Table 2).

3.2. Individual results and heat map

The heat-maps (Table 3A and 3B) display the Cunningham Panel results and the number of elevated tests before and after treatment are shown for each patient in this study. Elevated test values are highlighted in red; the intensity of color is indicative of magnitude of elevation above threshold. The count of elevated individual tests within each patient’s panels before and after treatment are indicated by blue and orange horizontal bars, respectively.
results pre- and post-treatment for all patients. Individual titers highlighted in red indicate a positive abnormal assay with the intensity of color indicating the extent of elevation. Titers without color highlighted indicate a normal assay value. The change in the Cunningham Panel results is highly associated with the reported change in symptoms following treatment as summarized in Table 4. The number of patients with elevated individual test results pre-treatment and post-treatment are shown in Fig. 3. The mean values for each of the individual tests in the Cunningham Panel before and after treatment are shown in Supplemental Fig. 1.

We evaluated the data for any association between changes in Cunningham Panel results and changes in patient symptoms after treatment or medical intervention using two distinct approaches: the Positive Test Count Method and the Magnitude of Change Method. Results from both analyses are summarized in the 2 × 2 contingency tables for sensitivity, specificity and accuracy (Fig. 4A and 5A), whereas the respective Receiver Operating Characteristic (ROC) curves are shown in Fig. 5A and 5B. The dot plots of individual scores by group and by analysis are shown in Fig. 4C and 5C.

**Fig. 3.** Number of patients with elevated test result.

**Fig. 4.** Results for the Cunningham Panel Test (Positive Test Count Score Method).

4A. Sensitivity and specificity of Cunningham Panel assays: 2 × 2 matrix and calculation of sensitivity, specificity and accuracy using Change in Number of Positive Tests ≥0.5 as predicting membership in Group 1 (optimum from ROC curve).

4B. Receiver Operating Characteristic (ROC) curve of Cunningham Panel assay results.

4C. Dot Plots for Change in Number Positive Tests. Blue dotted line (y = 0.5) shows threshold used in assigning membership in 2 × 2 matrix. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
3.2.1. Positive test count method

This method consists of assessing change in Cunningham Panel results (positive or negative) upon treatment by tallying the number of positive tests for each of the five assays. A 2 × 2 contingency table analysis (p = 5 × 10⁻⁸, Fisher exact test) revealed an 88% sensitivity, 83% specificity, and an accuracy of 86% (Fig. 4A). The ROC curve had an AUC of 93.4% (Fig. 4B). Dot plots of individual scores revealed good separation by group (Fig. 4C).

In the patients reporting symptom improvement following treatment, 88% (30/34) demonstrated a decrease in the number of individual positive Cunningham Panel tests. Within these patients, 62% (21/34) had all tests become negative whereas 26% (9/34) showed reduction in the number of positive tests and 12% (4/34) demonstrated an unchanged number of positive tests. No patients in the Improved/Resolved group had an increase in the number of positive tests post-treatment (Table 4). For patients whose symptoms failed to improve or worsened post-treatment (Group 2), 79% (19/24) demonstrated either the same number of positive tests pre-treatment or an increase in the number of positive tests in their panel. Within these patients, 54% (13/24) revealed an increase in the number of individual positive tests, with 25% (6/24) having an unchanged number of individual positive tests, and 8% (2/24) with a decrease in the number of positive tests in their panel.

3.2.2. Magnitude of change method

Using linear logistic regression we created an optimal function derived from the magnitude of change in the Cunningham Panel test scores which separated the groups of patients who responded to treatment vs those that did not respond. This multivariate method analysis creates a linear transformation which reduces the five individual values comprising the Cunningham Panel into one composite or combined score. Null deviance and residual deviance are 78.7 and 29.7 on 57 and 52 degrees of freedom, respectively. Accordingly, R² = 0.62. In contrast to linear regression, R² is not related to any correlation coefficient, nor is it a percentage of variance explained by the logistic model, rather it is a ratio indicating how close the fit is to being perfect (R² = 1) or...
the worst possible ($R^2 = 0$). As part of characterization of model quality, a summary of the values for all coefficients in "leave-one-out" analysis are shown in Supplemental Table 1. The probabilities for the misclassification in the original and leave-one-out validation are given in Supplemental Table 2.

Results in the 2 × 2 contingency table ($p = 6 \times 10^{-10}$, Fisher exact test) revealed an 88% sensitivity, 91% specificity with an overall accuracy of 90% (Fig. 5A). Values in 5A are taken from a threshold: $0.522 < \text{Probability} < 0.557$ which provides highest values per the ROC analysis in 5B. For example, if the threshold is set at 0.5, then the number of incorrect assignments of Group 2 to Group 1 increases from 2 to 3 of 24 whereas the number of incorrect assignments of Group 1 to Group 2 is unchanged at 4 of 34 (Supplemental Table 2) for an overall accuracy of 88%. The ROC curve used to define the threshold for predicting inclusion in Group 1 or Group 2 resulted in an AUC of 95.7% (Fig. 5B). Dot plots of individual scores revealed good separation by group (Fig. 5C). We find that there is good discrimination between Group 1 and Group 2 based on quantitative changes in the test results.

There is an interesting trend observed for patients in Group 1; CaMKII values were, more often than not, elevated prior to treatment and then significantly decreased in conjunction with a reduction in symptoms post-treatment ($p = 0.000004$), approaching levels associated with normal control populations (Fig. 6). In contrast, those patients in Group 2, defined as those whose symptoms did not improve post-treatment, tended to show elevated CaMKII levels both before and after treatment without any statistically significant change post-treatment ($p = 0.311$) (Fig. 7).

3.3. Summary of treatments and comparisons between groups

Retrospective evaluation of treatments revealed that of patients treated with immunotherapy (IVIg, plasmapheresis, Rituximab, or combination) 66.7% improved (Group 1), while just 33.3% failed to see improvement (Group 2). For patients who were treated with antimicrobial therapy alone, 70.6% of patients experienced symptom improvement vs. 29.4% failing to improve or worsening. Overall, treatment with multiple classes of therapeutic interventions in most cases showed modestly higher percentages of improvement, whereas in patients treated with psychotropic medications, dietary changes, or untreated, only 20% of patients reported symptom improvement. More specifically, four of four patients (100%) improved with combined IVIg and plasmapheresis, and one of two patients (50%) improved with combined IVIg and Rituximab. Only one of five (20%) patients improved who were treated with psychotropic medications, dietary changes, or no treatment (see Table 5). Note that in general, the utilization frequency of specific treatments is similar between Group 1 and Group 2 (Table 5). The use of “Immunotherapies Only” and “Antimicrobials Only” is somewhat higher in Group 1 than in Group 2, whereas the percentage of patients in Group 2 that received both classes of therapy is somewhat higher than in Group 1.

### Table 5

<table>
<thead>
<tr>
<th>Treatment Description</th>
<th>Group 1 Improved</th>
<th>Group 2 No improvement or worsened</th>
<th>% improved *</th>
</tr>
</thead>
<tbody>
<tr>
<td>A All therapies containing antimicrobials</td>
<td>19 (56%)**</td>
<td>13 (54%)</td>
<td>59.4%</td>
</tr>
<tr>
<td>B All therapies containing immunotherapies</td>
<td>21 (62%)</td>
<td>15 (63%)</td>
<td>58.3%</td>
</tr>
<tr>
<td>C Antimicrobials only</td>
<td>12 (35%)</td>
<td>5 (21%)</td>
<td>70.6%</td>
</tr>
<tr>
<td>D Immune therapies only</td>
<td>14 (41%)</td>
<td>7 (29%)</td>
<td>66.7%</td>
</tr>
<tr>
<td>Individual Immune therapies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVIg</td>
<td>9</td>
<td>5</td>
<td>64.3%</td>
</tr>
<tr>
<td>Plasmapheresis</td>
<td>1</td>
<td>1</td>
<td>50.0%</td>
</tr>
<tr>
<td>Rituximab</td>
<td>1</td>
<td>0</td>
<td>100.0%</td>
</tr>
<tr>
<td>Combination Immune therapies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVIg Plasmapheresis</td>
<td>4</td>
<td>0</td>
<td>100.0%</td>
</tr>
<tr>
<td>IVIg Rituximab</td>
<td>1</td>
<td>1</td>
<td>50.0%</td>
</tr>
<tr>
<td>E Antimicrobials + Immune therapies</td>
<td>9 (26%)</td>
<td>8 (33%)</td>
<td>52.9%</td>
</tr>
<tr>
<td>IVIg</td>
<td>6</td>
<td>7</td>
<td>46.2%</td>
</tr>
<tr>
<td>Plasmapheresis</td>
<td>0</td>
<td>1</td>
<td>0.0%</td>
</tr>
<tr>
<td>IVIg Plasmapheresis</td>
<td>3</td>
<td>0</td>
<td>100.0%</td>
</tr>
<tr>
<td>IVIg Plasmapheresis + Rituximab</td>
<td>0</td>
<td>1</td>
<td>0.0%</td>
</tr>
<tr>
<td>F Antimicrobials, immuno- or combination thereof</td>
<td>33 (97%)</td>
<td>20 (83%)</td>
<td>62.3%</td>
</tr>
<tr>
<td>G Other/none</td>
<td>1 (3%)</td>
<td>4 (17%)</td>
<td>20.0%</td>
</tr>
<tr>
<td>Psychotropic medications only</td>
<td>0</td>
<td>1</td>
<td>0.0%</td>
</tr>
<tr>
<td>Dietary changes only</td>
<td>1</td>
<td>1</td>
<td>50.0%</td>
</tr>
<tr>
<td>No treatment</td>
<td>0</td>
<td>2</td>
<td>0.0%</td>
</tr>
</tbody>
</table>
3.4. Reproducibility of ELISA testing in the Cunningham Panel

For repeated D1R assay we observed 207 readings at the same titer, 62 readings at one titer higher and 75 readings at one titer lower (Fig. 8), resulting in a distribution of 17.3%, 61.5% and 21.2%, respectively. No results were greater than one titer dilution away from the peak. When expanded to include mixed patient samples and additionally processed or concentrated patient samples, we saw rare instances (< 0.5%) where the assigned titer was two dilution steps from the peak. In no cases were any titers greater than two dilution steps from the peak.

3.5. Reproducibility of CAMKII assay results in the Cunningham panel

Seventeen samples underwent repeat CaMKII assay testing. The correlation between the original and the repeat test was 0.90 ($p < 1.67 \times 10^{-6}$) and the root-mean-square deviation (RMSD) is 13.8 (Fig. 8B). The deviation (Repeat Assay Value / Original Assay Value) was approximately 15% with no apparent dependence on the actual CaMKII activity over the range of values routinely measured. The baseline value by definition is 100, whereas the threshold for a positive in the CaMKII assay is set at 130 (30% above baseline values defined from measurements in pediatric controls).

In summary, repetitive testing of multiple samples, multiple times in the ELISA and CaMKII assays using patient samples demonstrates assay robustness and reproducibility.

4. Discussion

For PANDAS and PANS, there is a need to elucidate the pathophysiology and underlying disease mechanisms in order to improve the identification of patients, advance targeted therapies, and to clarify the characteristics of these disorders. The value of identifying a group of biomarkers that correspond to a disease etiology is that it can segment patients exhibiting similar symptoms into groups that can be administered different treatment modalities based upon their underlying disease etiology. In this 58 patient case series, we found that changes in assays of the Cunningham Panel parallel changes in patient symptoms following treatment. Using the change in the number of positive tests we were able to predict change in symptoms with an accuracy of 86%, a sensitivity of 88% and a specificity of 83%, respectively. Based upon the magnitude of change of individual test values of the Cunningham Panel, we were able to predict change in symptoms with an accuracy of 90%, a sensitivity of 88% and a specificity of 92%.

4.1. Comparison with other published studies

In a 2018 study of 80 patients having a diagnosis of ASD, in which 31 children with autoimmune encephalopathy received IVIg treatment, the Cunningham Panel predicted patient improvement and response to IVIg treatment with an accuracy of 81%, a sensitivity of 90% and a specificity of 67% based on the Aberrant Behavior Checklist (ABC) scores; with an accuracy of 88%, a sensitivity of 100% and a specificity of 75% based on the Social Responsiveness Scale (SRS) scores; and with an accuracy of 88% with a sensitivity of 100% and a specificity of 67% based on parental scores (Connery et al., 2018). The sensitivity and specificity of the Cunningham Panel in predicting IVIg responsiveness in children with autoimmune encephalopathy and diagnosed with ASD (81% to 88%) are similar to the performance accuracy we observed in this study (87% to 90%). Published literature reveals that children with ASD have strong family histories of immune dysregulation and inflammation, and that the literature supports a strong association of immune dysregulation and ASD (Rossignol and Frye, 2012). Because
current diagnostic criteria for ASD and PANDAS/PANS are based upon clinical symptom clusters (Chang et al., 2015), it is possible that a common underlying etiology of these disorders, or a subset of these clinical syndromes, may be immune dysregulation with antineuronal antibodies directed against the basal ganglia and/or other CNS targets in the brain.

A 2017 study questioned the clinical utility of the Cunningham Panel (Hesselmark and Bejerot, 2017a) based upon a retesting study of 53 patients, with 46 patients having a repeat test. The estimated sensitivity for individual tests ranged from 15% to 60% and the estimated specificity ranged from 28% to 92%. Shortly thereafter, the authors submitted a Corrigendum (Hesselmark and Bejerot, 2017b) acknowledging they had unknowingly utilized invalid blood collection tubes containing clot activators and serum separator gels (BD Vacutainer® SST™ II Advance tubes, Gold Top) which are not acceptable for collecting Cunningham Panel samples. Specimens collected in blood tubes containing excipients or additives have the potential for irreproducibility of results, potential interactions with patient’s specimens, and potential direct interference in the assays.

In the referenced study, the authors reported some “healthy controls” showing positive Cunningham Panel results. This could be a result of the invalid collection method or the exclusion and inclusion criteria utilized for their control population (Frye and Shimasaki, 2019). Family history of psychiatric, autoimmune, or movement disorder was not investigated in the controls nor was the history of recent, chronic or recurrent infections. Since PANDAS/PANS is often triggered by an infection which can be subclinical or occult, transient molecular mimicry could have confounded test results. More importantly, although the patients were asked about a psychiatric or autoimmune diagnosis, symptoms of autoimmune or psychiatric disorders were not specifically investigated. This lack of detailed screening of the controls calls into question the extent to which the controls were verified as healthy.

4.2. Therapeutic interventions

Although this current study was not designed as a comparison of treatment interventions, within those whose symptoms improved post-treatment, a greater percentage received some form of immunomodulatory or antimicrobial therapy alone, compared to the patient group whose symptoms did not improve. It was observed that a higher percentage (70.6%) of patients improved who were treated with antimicrobial therapy alone, whereas 52.9% of patients treated with both modalities saw improvement. It is unknown whether those patients who required multiple therapeutic modalities may have had longer duration of illness or greater severity of symptoms. PANDAS/PANS patients typically have undergone multiple symptomatic-focused treatments prior to a recognition of their underlying infectious trigger and autoimmune etiology. It may be that prolonged duration of these conditions prior to proper diagnosis and treatment could impact the outcome of a course of therapy and the treatment duration required to observe symptom resolution in these patients.

A confounding issue that could potentially influence the outcome of certain immunomodulatory treatments with IVlg is the observation that in some IVlg lots we tested, we find varying, but significantly elevated sizes of patients in each group, which was limited to the numbers of patients who required multiple therapeutic modalities may have had longer duration of illness or greater severity of symptoms. PANDAS/PANS patients typically have undergone multiple symptomatic-focused treatments prior to a recognition of their underlying infectious trigger and autoimmune etiology. It may be that prolonged duration of these conditions prior to proper diagnosis and treatment could impact the outcome of a course of therapy and the treatment duration required to observe symptom resolution in these patients.

A confounding issue that could potentially influence the outcome of certain immunomodulatory treatments with IVlg is the observation that in some IVlg lots we tested, we find varying, but significantly elevated levels of antineuronal antibodies against the biological targets and CaMKII activation in the Cunningham Panel (data not shown). Because IVlg is a product produced through the concentration of immunoglobulins from 1000 or more patients, it would not be unexpected to observe this. However, it is key that post-treatment testing with the Cunningham Panel be performed after significant clearance of potential exogenous antineuronal antibodies, as this may confound patient results. Estimates of half-life of some manufacturers’ IVlg are as long as 40 days in patients (IVlg Manufacturer’s Package Insert). Also, in a research setting where testing was performed at regular intervals during immunomodulatory treatment, completely normal testing results have been observed, due to the possibility of immunomodulatory treatment interfering with testing results, either by suppressing the production of these autoantibodies or direct interference. Because of potential for immunomodulatory treatment interference with test results, prescribers are advised to consider waiting at least six to eight weeks or longer following treatment to determine treatment effectiveness in reducing these antineuronal antibodies. Within this study the mean time from pre-treatment testing to post-treatment testing was 68.1 and 66.2 weeks respectively for Group 1 and Group 2.

4.3. Future directions

There is a clear need for more treatment practice guidance for post-infectious autoimmune neuropsychiatric disorders. The recently published treatment guidelines for patients with PANS/PANDAS (Swedo et al., 2017; Thiemann et al., 2017; Frankovich et al., 2017; Cooperstock et al., 2017) and clinical and treatment information on the PANDAS Physician Network (PPN) (‘PANDAS Physician Network’, 2018) will help establish a standard for treatment of these patients. Additionally, in order to help guide treatment decisions, we are systematically analyzing our biobank of over 8000 annotated samples to study how baseline data may help stratify patient populations into diagnostic subgroups for predicting treatment response.

Other clinical reports have been published showing effective utilization of immunomodulatory treatment for autoimmune encephalitis based upon the presence of antineuronal antibodies directed against NMDA receptor (Dalmou et al., 2017), voltage-gated potassium channel-complex (VGKC) (Vincent et al., 2011), leucine-rich glioma inactivated-1 (LGII), astrocyte aquaporin-4 (AQP4), (Zékeridou and Lennon, 2015), glutamic acid decarboxylase (GAD65), gamma-aminobutyric acid-B receptor (GABAB), and others (Mader et al., 2017; Platt et al., 2017; Mohammad and Dale, 2018; Lancaster, 2016; Dale et al., 2017). It is plausible there could be additional antineuronal antibody targets that lead to symptoms of autoimmune encephalitis. Interestingly, in a study of 61 patients diagnosed with autoimmune encephalitis by confirmatory testing such as MRI, CSF inflammation, EEG and FDG-PET/CT, it was determined that the anti-neuronal antibodies in this referenced study, such as those listed above, did not account for 48% (29/61) of these patients presenting with autoimmune encephalitis (Probascos et al., 2017). A future study will include the examination of a broader range of anti-neuronal antibodies. Thus, while there are many known targets for autoantibodies in patients with autoimmune encephalopathies, antibodies to extracellular epitopes of dopamine D2 receptor have also been identified in patients with pediatric basal ganglia encephalitis (Dale et al., 2012) a related disorder with overlapping yet distinct clinical presentation to PANDAS/PANS.

5. Study limitations

Pre- and post-treatment symptom responses were carefully collected and documented by questionnaire and telephone follow-up with parents and physicians. However, because of the lack of a standardized instrument and a single individual assessing symptom severity in all these patients, there could be inter-patient variation in the self-reporting of symptom severity. Future studies would benefit from the utilization of a standardized rating scale in a single study utilizing a single independent assessor for measuring the severity of symptoms. Since PANS/PANDAS is a diagnosis of exclusion and patients currently may not receive this diagnosis during a primary medical visit, patients that are referred for testing may have visited multiple clinicians and received multiple therapies that have failed prior to their Cunningham Panel. Thus, there might be significant variability between symptom onset and initial testing with the Cunningham panel.

Other limitations to this study include the relatively small sample sizes of patients in each group, which was limited to the numbers of
patients that had pre- and post-treatment testing during this interval, and the relatively small number of carefully screened pediatric healthy controls from which the normal or basal levels were obtained.

Although published literature has demonstrated that anti-infectives and immune modulatory therapy can indeed be effective in PANDAS patients (Kovacevic et al., 2015; Perlmutter et al., 1999), the exact treatment used, dose and duration of treatment and when the treatment was instituted was not controlled as part of this study and could have added to variability.

Interestingly, autoantibodies against certain biomarkers have also been identified in other inflammatory, movement, and/or neuropsychiatric conditions. Although autoantibodies against any individual target may not be diagnostic for PANDAS/PANS or Sydenham’s chorea, the entire panel taken together may be a strong aid in a physician’s clinical diagnosis of an autoimmune etiology. There also remains the possibility that autoantibodies against these targets may not necessarily be pathogenic but rather an epiphenomena associated with this disorder. Further research and clinical studies are in progress to assess this as a causal contribution or a downstream result of the disorder.

6. Conclusions

We report here for the first time a strong positive association between the change in anti-neuronal antibody titers and antibody-mediated CaMKII activation measured as part of the Cunningham Panel and change in neuropsychiatric symptoms in patients with PANDAS/PANS. These data provide supportive evidence that the change in levels of serum autoantibodies directed against neuronal pathophysiological processes may correlate with the change in neuropsychiatric symptoms in patients with certain neuropsychiatric disorders.

These observations and results support a potential common proposed mechanism where infection-triggered immune dysregulation may lead to antineuronal antibodies directed against specific targets in the brain resulting in neuropsychiatric symptoms (Labrie and Brundin, 2019). A Danish study by Köhler-Forsberg et al. of over one million individuals provides compelling epidemiologic evidence that severe infections are linked to the onset of neuropsychiatric illnesses in children (Köhler-Forsberg et al., 2019). They observed an 8-fold increased risk for obsessive-compulsive disorder in teenagers, and the study identified a 1.5-fold to 5.6-fold elevated risk for neurodevelopmental delay, mental retardation, and behavioral and/or emotional disturbances in the young. If this mechanism proves to be true, there is the potential that subsets of other clinically-defined neuropsychiatric disorders and conditions may have a pathophysiology representing a broader category of disorders referred to as “Autoimmune Encephalopathies of Infectious Etiology,” (Fig. 9 Graphical Abstract).

In an era of precision medicine, there would be clinical advantages if targeted testing could segment patients having heterogeneous symptoms into discrete groups based upon responsiveness to a particular treatment modality. Although the historical identification of the anti-neuronal antibodies in the Cunningham Panel originated from patients diagnosed with Sydenham’s chorea and later PANS/PANDAS patients, these biomarker targets may help identify broader populations of patients experiencing neuropsychiatric symptoms triggered by a similar autoimmune etiology that may be responsive to immune modulation therapy. Testing results may also provide a clinician with the biological evidence for the diagnosis of an immune-mediated disorder which may alter their treatment modality.

Based upon this retrospective analysis, there is evidence to support that the Cunningham Panel may have value as an aid in a clinician’s diagnosis and management of patients with PANDAS/PANS. Although it is not clear whether the presence of antineuronal antibodies to these targets may be causal or an associative response to a patient’s neuropsychiatric symptoms, identifying such an objective measure is essential for performing future studies on treatment outcomes and to help elucidate a disease mechanism. The results of this study represent a step towards validating antineuronal antibody tools that may be utilized as an aid in a physician's diagnosis of PANDAS/PANS and support future prospective studies into the understanding of its etiology and pathogenesis. The validation of biological markers may also lead to the identification and development of more efficacious targeted therapeutics for treating patients with these neuropsychiatric disorders. Further, the data generated in this study suggests a larger analysis is warranted including more patients to explore whether baseline data can predict treatment response in patients with neuropsychiatric symptoms.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.577138.

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Author Disclosures

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