Multifactorial analysis of opsoclonus-myoclonus syndrome etiology (“Tumor” vs. “No tumor”) in a cohort of 356 US children

Michael R. Pranzatelli | Elizabeth D. Tate | Nathan R. McGee

National Pediatric Myoclonus Center, National Pediatric Neuroinflammation Organization, Inc., Orlando, Florida, USA

Correspondence
Dr. M. R. Pranzatelli, National Pediatric Neuroinflammation Organization, Inc., 12001 Research Parkway, Suite 236, Orlando, FL 32826, USA. Email: mpranzatelli@omsusa.org

Contact information for co-authors
Elizabeth D. Tate, M.N., ARNP, FNP-C, National Pediatric Neuroinflammation Organization, Inc. 12001 Research Parkway, Suite 236, Orlando, FL 32826, USA. Email: omsusa@omsusa.org

Nathan R. McGee, B.S. National Pediatric Neuroinflammation Organization, Inc. 12001 Research Parkway, Suite 236, Orlando, FL 32826, USA. Email: mcgee6510@att.net

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Abstract

Background: Pediatric opsoclonus-myoclonus syndrome (OMS) presents a paradox of etiopathogenesis: A neuroblastic tumor (NB) is found in only one half of the cases, the others are ascribed to infections or designated as idiopathic.

Method: From an IRB-approved observational study of 356 US children with OMS, secondary analysis of “etiology” and related factors was performed on a well-characterized cohort. The “Tumor” (n = 173) and “No Tumor” groups (n = 183), as defined radiologically, were compared according to multiple factors considered potentially differentiating. Data were analyzed retrospectively using parametric and nonparametric tests as indicated.

Results: Patients with NB were not distinguishable by prodromal symptoms, OMS onset age, gender, race/ethnicity, OMS severity, rank order of neurological sign appearance, or geographic distribution. Various CSF immunologic biomarker abnormalities of OMS did not vary in the presence or absence of a detectable tumor: frequency of six lymphocyte subsets, or concentrations of 18 cytokines/chemokines, cytokine antagonists, chemokine receptors, cell adhesion molecules, or neuronal/glial markers. Prior responsiveness to conventional immunotherapy was not contingent on tumor/no tumor designation.

Conclusions: Multiple convergent factors provide compelling empirical evidence and rationalize the concept that OMS is one neurological disorder, regardless of apparent etiology. Limitations to the current clinical etiologic classifications as paraneoplastic, parainfectious/post-infectious, and idiopathic etiology require antigen-based biological solutions to tease out the molecular pathophysiology of viral/tumoral mechanisms. Systematic studies, regardless of presumed etiology, will be necessary to find the highest-yield combination of imaging approaches, screening for infectious agents, and new biomarkers. Two testable hypotheses for future research are presented.

KEYWORDS
ganglioneuroblastoma, ganglioneuroma, neuroblastic tumors, neuroblastoma, neuroblastoma regression, OMA, paraneoplastic syndrome, pediatric opsoclonus-myoclonus syndrome

1 | INTRODUCTION

Opsoclonus-myoclonus syndrome (OMS), alias opsoclonus-myoclonus-ataxia syndrome, is an inflammatory neurologic syndrome with a strong connection to cancer.¹⁻³ Tumors are usually neuroblastic (NB), residing in the body cavity, inclusive of neuroblastoma, ganglioneuroblastoma, and ganglioneuroma.⁴ The favorable prognosis of OMS-associated NB has been attributed to unique tumor biology (lower-risk/more mature tumors, lack of MYCN amplification, and aneuploid DNA content) and tumor/host factors (increased presence of lymphoid infiltrates/follicles).⁵,⁶ even when the genomic profile of NB is unfavorable.⁷

Abbreviations: CCL, C–C motif chemokine ligand; CD, cluster of differentiation; CSF, cerebrospinal fluid; CXCL, C–X–C motif chemokine ligand; IL, interleukin; MIBG, metaiodobenzylguanidine; NB, neuroblastoma; NMDAR, N-methyl-D-aspartate receptor; NT, neuroblastic tumor; OMES, Opsoclonus-Myoclonus Evaluation Scale; OMS, opsoclonus-myoclonus syndrome; TS, total score

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The diagnostic paradox is that a paraneoplastic etiology can only be proven presently in about 50% of the cases. One proposed explanation is missed tumors. Compared to their often-aggressive counterparts without OMS, NB can be more difficult to detect in OMS due to a lower frequency of metaiodobenzylguanidine (MIBG) uptake and urinary catecholamine secretion. These differences may elude or delay diagnosis. NB, arising from the developing sympathetic nervous system, may also regress spontaneously. Although OMS etiology has been defined largely through diagnostic tumor imaging studies, there is no consensus on the optimal type, number, or frequency of scans.

An alternative explanation is that tumors are not the only provocation for OMS. Imaging-negative cases have been designated as "parainfectious," "post-infectious," or "idiopathic," usually on the basis of whether "prodromal" (preneurological) symptoms were interpreted as suggestive of infection. Despite a litany of infections reported in case studies, some with intrathecal inflammation, no consistent pathogen has been identified, the infection is usually nondeclaratory (nondemonstrable), and patients may have no "infectious" prodrome.

The biological validity of OMS etiologic designations has been enigmatic and difficult to address. Are there two portals of entry to a uniform pathogenesis or two separate but equal pathways with shared downstream events? Besides being intellectually dissatisfying and confusing, this dichotomization has practical consequences for the extent of diagnostic testing, the treatment of OMS, and which type of specialist handles the case. Lack of data in this rare disorder has resulted in an inability to systematically classify patients, frame viable hypotheses, and rethink strategies for translational research on the issue.

The present study accepts the compelling reason and unique opportunity to study multiple factors that may be involved. To approach the problem, we performed extensive secondary analysis of just the US cases of a well-defined population of pediatric-onset OMS. Primary analysis of the data, which did not address this issue, is not reiterated here. Demographic, neurologic, oncologic, epidemiologic, neuroimmunologic, and neuropharmacologic data were examined in an effort to differentiate the purported OMS etiologies in the largest US patient population yet applied for this purpose. The main questions were: (1) is OMS phenotypically different depending on the perceived etiology?: (2) what is the evidence for or against a true etiologic dichotomy?: and (3) what approaches would be required to reach a definitive answer? The present report extends knowledge about OMS in the aggregate, including our experience with its neuroinflammation which involves immune cells and mediators that foster the neogenesis of lymphoid follicles.

2 METHODS

2.1 Participants

The objective of the study was to compare patients with a demonstrated NB ("Tumor") with those in whom no tumor was found ("No Tumor"). Cross-sectional clinical and demographic features, OMS onset age distribution, prodromal symptoms, time to OMS diagnosis, OMS duration, geographical distribution, ranked order of neurologic signs, neuroimmunologic markers in CSF and blood, and early neuropharmacologic responses were evaluated. This was a monocenter, observational study analyzed retrospectively. All patients had been evaluated by the first two authors at the National Pediatric Myoclonus Center specializing in pediatric-onset OMS. The OMS population, a representative cohort, comprised 356 children with clinically confirmed OMS. Data were collected previously by written parental consent for the IRB-approved study SIU SOM, Springfield, IL. Additionally, Western IRB (Puyallup, WA) granted permission for retrospective analysis. Secondary analysis of the data was used to address the question of OMS etiology with literature as supporting documents. The opsoclonus-myoclonus evaluation scale (OMES) used for assessing OMS severity is reprinted in Supplementary Table S1. Extensive methodological details were provided with the primary analysis.

2.2 Tumor screening

NB type, location, and stage are shown in Supplementary Table S2. We did not have access to formal risk classification, but 93% of the patients had stage 1 or 2 tumors; 7%, stage 3 or 4.

At the time of our evaluation, most patients had been screened for NB, but the types and number of tests were not uniform. Among them were urinary catecholamines; CXR and abdominal ultrasound; MRI of neck, chest, abdomen, and pelvis; CT with oral and intravenous contrast; and MIBG scan. If a tumor had not been found, screening was broadened to include tests not performed previously, making sure both anatomic imaging methods (CT, MRI) and functional imaging scintigraphy ([123I]MIBG) had been employed.

It was also our standard practice (not result of study) to recommend MRI reimaging at 6-month intervals for 2 years after OMS onset in imaging-negative cases, and at the time of unexplained OMS relapse or failure to remit on immunotherapy. Patients already shown to have a NB were monitored by their oncologist for tumor recurrence per standard of care.

Possible under-reporting of late tumor detection by parents over the years was anticipated. The majority of families stayed in touch with us, but it was not possible to contact every family to determine if a tumor was subsequently detected in tumor-negative cases.

Commercial screening for "paraneoplastic" autoantibodies and antibodies associated with autoimmune encephalitis was so seldom positive, results were not dichotomized.

2.3 Laboratory procedures for neuroinflammation detection

2.3.1 Lymphocyte subset analysis

Freshly collected CSF (on ice) and blood samples were brought to the clinical flow cytometry laboratory for processing. Spun leukocytes were labeled using a panel of directly conjugated monoclonal antibodies to surface adhesion proteins in combination with anti-CD45 and anti-CD3. Lymphocyte subsets included total B cells...
(CD3-CD19+) and T cells (CD3+), T helper/inducer cells (CD3+CD4+), T cytotoxic/suppressor cells (CD3+CD8+), gamma delta T cells (TCRγδ+CD3+), and natural killer cells (NK or CD3-CD16/56+). Percent of lymphocytes indicates percent of positive cells. Further methodologic details and our data from non-inflammatory pediatric neurological controls have been reported previously.26,37 Dichotomized flow cytometric data on the CXCR3+CD4+ T-cell receptor43 are shown in Supplementary Table S3.

### 2.3.2 Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assays (ELISA) were used to measure the cytokines B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL), C–C motif chemokine ligands 19 (CCL19) and 21 (CCL21), as well as the C–X–C motif chemokine ligands 10 (CXCL10), 12 (CXCL12), and 13 (CXCL13). Assay kits were purchased from R&D Systems (Minneapolis, MN), except for the APRIL kit, which was purchased from eBioscience (Vienna, Austria). Corresponding control data from the principal investigator’s laboratory have been published.42,43,45,47 Our dichotomized data on ELISA-measured Th2 chemokines (CCL17, CCL22),44 soluble cell adhesion molecules (sICAM-1, sVCAM-1),39 and neuronal/glial markers (NFL, GFAP)40 are presented in Supplementary Table S3.

### 2.3.3 Multiplexed fluorescent bead-based immunoassays

Multiplexed fluorescent bead-based immunoassays, utilizing the Luminex 100 LabMAP system (Luminex Co., Austin, TX), were performed to measure cytokines and chemokines in CSF and serum samples aliquoted and stored at −80 °C in the National Pediatric Myoclonus Center biorepositories. Assays were run in batches in triplicate using a combination of 22-plex and 12-plex Beadlyte Human Cytokine Detection kits (Upstate, Lake Placid, NY) as described previously.39 Analyses comprised cytokines, such as interleukin-6 (IL-6), IL-12p40; IL-16, IL-8 (aka CXCL8); the IL-1 receptor antagonist (IL-1Ra) and IL-2 receptor antagonist (sIL-2Ra); and chemokines, including the C–C motif chemokines ligand 2 (CCL2), CCL4, and CCL5, and growth-related oncogene (CXCL1). Methodological details and our control data are available elsewhere.39,43

### 2.4 Statistical analysis

Mapland Spreadsheet Mapping Software (Pleasanton, CA) was used for geographical data analysis. With GraphPad Prism Version 7.02 (San Diego, CA), statistical comparisons were made between children with demonstrated NB and those testing negative. For pairwise comparison of continuous variables, two-tailed t-tests were used. ANOVA was implemented in the analysis of OMS severity category (mild, moderate, severe), duration category (acute, subacute, chronic), and treatment category (untreated, currently treated at evaluation, or previously—not currently—treated). Dunn post-hoc tests were used. For frequency analysis, Chi-square or Fisher Exact test was used. Data on the parent-numbered order of presenting neurological signs were ranked because one number is dependent on the next, not all signs are present in every patient, and the number of parents responding varied.

A P-value < 0.05 was considered statistically significant throughout. Bonferroni corrections were made for multiple corrections (α at 0.05/n of comparisons).

### 3 RESULTS

#### 3.1 Demographic characteristics of OMS with and without tumor

The cardinal demographic characteristics are shown in Table 1. Patient age was wide-ranging but well balanced between groups, with no statistically significant differences in means/medians. The racial/ethnic demography for children with a demonstrated NB did not differ statistically from those without. Patient gender frequency also did not discriminate the two groups, though there appeared to be a slightly greater female predominance in the tumor group.

#### 3.2 Neurological severity by perceived OMS etiology

Mean total score (TS) of the OMES fell within the moderate severity category of 13–24 and was not significantly different between groups (Table 1). ANOVA revealed a comparable statistically significant effect of OMS duration category on TS in the Tumor (P < 0.0001) and No Tumor groups (P < 0.0001), but no significant effect of perceived OMS etiology. The proportion of OMS duration categories in the two groups was very similar.

#### 3.3 Onset-age distribution of OMS with and without tumor

There were no statistically significant differences in the mean OMS age-of-onset frequency (Fig. 1). Both groups showed the greatest prevalence between the ages of 1–2 years. The rise in frequency was steeper on the left side of the peak age; and decline in frequency on the right was more gradual, with a low frequency after the age of 4.5–7.5 years. In a month-by-month breakdown of children with OMS over the first 2 years (Fig. 1, inset), both etiologies were represented. OMS onset age was slightly higher in the No Tumor group, owing to late-onset cases. To determine the timing of the difference, age cut-offs were analyzed. Using a cut-off of ≤2 years, the OMS onset age was not statistically different in the two groups: 5.5 ± 4.6 months (n = 131, Tumor); 5.2 ± 4.4 (n = 126, No Tumor). It was no different with the age cut-off of <4 years either: 1.6 ± 0.7 years (n = 169); 1.7 ± 0.7 (n = 167), respectively. The difference became significant at the age cut-off of ≤5 years due to late onset cases primarily in the No Tumor group.

#### 3.4 Prodromal symptoms by perceived OMS etiology

Prodromal symptoms were similar in type and frequency between the two groups (Fig. 3A). Fever and ear infection did not discriminate the groups. The most prevalent symptoms, crying and irritability, were general in nature. Neither vomiting/diarrhea (possibly suggestive of
### TABLE 1
Cross-sectional comparison of clinical and demographic features of OMS based on tumor detection

| Feature                          | Tumor detected | No tumor detected | P  |
|----------------------------------|----------------|-------------------|----|
| N                                | 173 (49%)      | 183 (51%)         |    |
| Age at evaluation, years         | 3.9 ± 3.5      | 3.7 ± 3.6         | 0.58 |
| Age at OMS onset, years          | 1.7 ± 0.89     | 2.1 ± 1.4         | 0.17 |
| OMS duration, years              | 2.3 ± 3.6      | 1.6 ± 3.1         | 0.08 |
| OMS duration category, n         |                |                   | 0.08 |
| Acute (0–3 months)               | 31 (19%)       | 48 (28%)          |    |
| Subacute (3–12 months)           | 57 (35%)       | 64 (37%)          |    |
| Chronic (>12 months)             | 73 (45%)       | 60 (35%)          |    |
| TS versus duration category      |                |                   |    |
| Acute                            | 20.7 ± 8.4     | 20.6 ± 8.3        | 0.99 |
| Subacute                         | 16.0 ± 8.5     | 16.6 ± 8.1        | 0.69 |
| Chronic                          | 11.9 ± 7.5     | 12.8 ± 8.6        | 0.52 |
| OMS severity (TS)                | 15 ± 8.7       | 16.5 ± 8.8        | 0.52 |
| OMS severity category, n         |                |                   | 0.46 |
| Mild (TS 0–12)                   | 64 (40%)       | 51 (27%)          |    |
| Moderate (TS 13–24)              | 71 (45%)       | 45 (38%)          |    |
| Severe (TS 25–36)                | 23 (15%)       | 22 (19%)          |    |
| Time to OMS diagnosis (months)   | 2.1 ± 3.2      | 3.0 ± 4.5         | 0.12 |
| Time to diagnosis category, n    |                |                   | 0.12 |
| <1 months                        | 51 (57%)       | 40 (42%)          |    |
| 1–3 months                       | 22 (24%)       | 32 (33%)          |    |
| 3–6 months                       | 12 (13%)       | 11 (11%)          |    |
| 6–12 months                      | 5 (6%)         | 11 (11%)          |    |
| >12 months                       | 0              | 2 (2%)            |    |
| Gender, n                        |                |                   | 0.09 |
| Male                             | 69 (40%)       | 89 (49%)          |    |
| Female                           | 104 (60%)      | 94 (51%)          |    |
| Racial/ethnic demography, n      |                |                   | 0.66 |
| White, non-Hispanic              | 129 (75%)      | 128 (70%)         |    |
| Hispanic/Latino                  | 24 (14%)       | 27 (15%)          |    |
| Black                            | 9 (5%)         | 15 (8%)           |    |
| Asian/Oceanic                    | 1 (0.5%)       | 4 (2%)            |    |
| American Indian                  | 1 (0.5%)       | 1 (0.5%)          |    |
| More than one race               | 9 (5%)         | 8 (5%)            |    |
| OMS relapse history, n           |                |                   | 0.39 |
| Yes                              | 76 (44%)       | 71 (39%)          |    |
| No                               | 97 (57%)       | 111 (61%)         |    |
| Parental age at conception, y    |                |                   |    |
| Mother                           | 28 ± 6         | 29 ± 6            | 0.25 |
| Father                           | 31 ± 7         | 31 ± 6            | 0.91 |

*At time of initial evaluation by our center.

Total score (TS): n = 152 for tumor, 149 for no tumor.

Group means are given with SD. There were no statistically significant differences between groups per t-tests.

N values are given with percentages. Chi-square or Fisher exact test revealed no significant inter-group differences.
gastrointestinal infection), nor cough/fever (possibly suggestive of upper respiratory infection) had group-discriminating value.

In the Tumor group, specific infections recalled by parents in the weeks immediately preceding OMS onset included: "rotavirus" (n = 2), "Coxsackie" (n = 1), "chickenpox" (n = 2), "croup" (n = 1), "flu" (n = 1), "thrush" (n = 1), "laryngitis" (n = 1). In the No Tumor group, there was "pink eye" (n = 1), "strep throat" (n = 1), "wheezing" (n = 1), and "upper respiratory infection" (n = 1).

3.5 | Time to OMS diagnosis

The average time to diagnosis of OMS, which varied widely, did not differ statistically in the two groups. When clinically convenient cut-points were assigned, about one-half of the patients were diagnosed within the first month and three quarters by 3 months, but one-fifth were diagnosed only in the second half of the first-year post-onset. OMS etiology had no statistically significant effect in this frequency analysis.

3.6 | OMS duration

There was no statistically significant effect of apparent OMS etiology on mean OMS duration.

3.7 | Geographic distribution of OMS cases identified as tumor versus no tumor

The data were analyzed for geographical or latitudinal patterns. By visual inspection, there was no apparent difference in the geographical distribution of patients with tumor versus no tumor (Fig. 2). There was an admixture in populated areas, and, in other areas, the distribution seemed random and not mutually exclusive. There was no obvious difference in designated tumor cases in rural versus metropolitan areas. Also, there were no latitudinal differences in geographic distribution, which might be found for some vectors, between the two groups.

3.8 | Ranked order of neurologic signs

The order of appearance of 10 neurological signs was statistically significant for Tumor (P < 0.0001) and No Tumor groups (P < 0.00001). In both, signs of gait ataxia (staggering and falling) were earliest. Although the same kinds of neurological signs were present in both groups, there was no statistically significant inter-group difference in frequencies (Fig. 3B).

3.9 | Relapse history

Based on OMS relapse history at the time of evaluation, there were more nonrelapsers than relapsers in either group. However, the proportion of patients with and without relapse was not significantly different in the Tumor and No Tumor groups.
FIGURE 3  (A) Frequency of prodromal symptoms of OMS per parents in patients with and without tumor. There were no statistically significant differences between groups. (B) Order of appearance of neurological symptoms of OMS per parents in patients with tumor versus no tumor. Data shown are medians with IQR. There were no statistically significant differences between the two groups.

3.10 | Neuroimmunologic markers

The results of assays for CSF lymphocyte subsets (immunphenotype), chemokines and other cytokines, and brain-related proteins in OMS were compared in the two groups (Table 2). No statistically significant group differences were found after Bonferroni corrections for multiple comparisons. The few percent differences in the frequency of certain immune cells are not biologically relevant, as they fall within the variance of the flow cytometer.

CSF B-cell expansion and reduction in the CD4/CD8 ratio were comparable in the Tumor and No Tumor groups. The frequencies of NK cells and γδ T cells were within the control range. In blood, lymphocyte subset frequencies did not differ between OMS and controls.

CSF concentrations of a panel of chemokines, other cytokines, cytokine receptor antagonists, cell-adhesion molecules, and neuronal/glial markers did not differentiate perceived etiologic groups.

3.11 | Early neuropharmacologic responses by history

The proportion of untreated, currently treated (on arrival), and previously (not currently) treated subgroups was comparable in the Tumor and No Tumor groups (Fig. 4A). The majority of patients were on immunotherapy at the evaluation; some were polymedicated with conventional agents. There were 141 treated patients in the Tumor group (87%) and 118 in the No Tumor group (73%). There was a statistically significant difference between the two groups only in the number of agents the patients had received ($P = 0.002$, Chi-square). In the Tumor group, 18.5% had received a single agent; 81.5% received two or more agents. In the No Tumor group, 36.5% had received monotherapy, and 63.5% received two or more agents.

As anticipated, TS was significantly higher in untreated than treated OMS (Fig. 4B), however, mean total scores of treated patients were in the moderate severity range whether patients arrived on immunotherapy or had only received it previously, despite the fact that OMS duration was longer in the previously treated group (Fig. 4C). No significant effect of OMS etiology was found.

The time to treatment appeared slightly longer for ACTH than for IVlg and prednisone, but was comparable in Tumor and No Tumor groups (Fig. 4D). The frequency of “dramatic response” to therapy with prednisone, ACTH, or IVlg was analyzed (Fig. 4E). More patients were reported by parents to respond to ACTH (80%) than to prednisone or IVlg (each 50%), but there was no apparent effect of OMS etiology on the response.

TS was significantly lower (~22%) in patients treated with two or more agents than monotherapy ($P = 0.002$) when treated OMS were combined, but the statistical significance was less in group analysis (Fig. 4F).

4 | DISCUSSION

The main finding of this population-based study was that neurologic, oncologic, epidemiologic, neuroimmunologic, and neuropharmacologic data in the largest reported cohort of children with OMS failed to differentiate OMS in the group designated as “paraneoplastic” from that designated as “para/post-infectious” or “idiopathic.” Barring demonstration of the tumor, resection scar, or visible tumor or surgical complications, such as Horner syndrome, the clinical phenotype of the groups was indistinguishable. Perceived OMS etiology had no bearing on the OMS signs, as speculated previously in some small case series.

Because the literature offers incomplete and sometimes conflicting evidence on this point, the present study, which encompasses the contradictions, makes a contribution.

Interpretation should be mindful that the conclusions of the current study pertain to children up to the age of 9.8 years, which is also the main prevalence of NB (90% diagnosed by the age of 5 years; rare in people > 10 years). OMS occurrence in adolescence, which is uncommon, cannot be addressed by these data. Infections with a self-limited course have been reported in tweens and teens, and data on OMS in that late-onset age group are too scant to extrapolate.
### TABLE 2  Cross-sectional comparison of neuroinflammatory markers based on tumor detection in OMS

| Marker                  | CSF Tumor       | CSF No tumor     | P     | Blood/serum Tumor   | Blood/serum No tumor | P     |
|-------------------------|----------------|-----------------|-------|---------------------|----------------------|-------|
| **Leukocytes/cu mm**    | 2.1 ± 2        | 2.7 ± 3         | 0.11  | 22 ± 11             | 23 ± 10              | 0.52  |
| **Lymphocyte subsets**  |                |                 |       |                     |                      |       |
| % B cells               | 3.4 ± 3        | 4.1 ± 3         | 0.08  | 22 ± 11             | 23 ± 10              | 0.52  |
| % CD3+ T cells          | 86 ± 7         | 84 ± 7          | 0.04  | 63 ± 10             | 63 ± 11              | 0.97  |
| % CD4+ T cells          | 47 ± 11        | 47 ± 12         | 0.81  | 34 ± 8              | 36 ± 10              | 0.21  |
| % CD8+ T cells          | 32 ± 9         | 30 ± 9          | 0.08  | 24 ± 7              | 21 ± 6               | 0.01  |
| % γδ T cells            | 10 ± 7         | 10 ± 8          | 0.63  | 8.7 ± 5             | 8.5 ± 4              | 0.78  |
| % NK cells              | 4.6 ± 3        | 5.3 ± 4         | 0.07  | 12 ± 8              | 11 ± 6               | 0.47  |
| **Chemokines**          |                |                 |       |                     |                      |       |
| Primarily B cell        |                |                 |       |                     |                      |       |
| [CXCL13] pg/ml          | 8.7 ± 20       | 11 ± 26         | 0.37  | 110 ± 53            | 110 ± 49             | 0.98  |
| [CCL19] pg/ml           | 34 ± 55        | 38 ± 58         | 0.52  | (168) (169)         | (134) (129)          |       |
| Primarily Th1 cell      |                |                 |       |                     |                      |       |
| [CXCL10] pg/ml          | 805 ± 1118     | 846 ± 1227      | 0.75  | 156 ± 140           | 149 ± 113            | 0.69  |
| [CXCL12] pg/ml          | 449 ± 294      | 443 ± 287       | 0.86  | 1586 ± 443          | 1473 ± 337           | 0.17  |
| [CXCL9] pg/ml           | 69 ± 91        | 61 ± 69         | 0.54  | (122) (117)         | (48) (37)            |       |
| [CXCL8] pg/ml           | 15 ± 12        | 13 ± 9          | 0.26  | (66) (69)           | (82) (85)            |       |
| [CCL21] pg/ml           |                 | 496 ± 170       | 0.35  | (98) (98)           | (98) (98)            |       |
| [CXCL1] pg/ml           | 31 ± 26        | 29 ± 20         | 0.58  | 1652 ± 800          | 1726 ± 826           | 0.61  |
| [CCL4] pg/ml            | 29 ± 69        | 22 ± 46         | 0.30  | (64) (69)           | (57) (73)            |       |
| [CCL2] pg/ml            | 389 ± 219      | 374 ± 239       | 0.68  | 93 ± 115            | 103 ± 165            | 0.70  |
| [CCL5] pg/ml            | 5.0 ± 10       | 5.3 ± 12        | 0.87  | 1042 ± 500          | 1186 ± 627           | 0.24  |
| Cytokines               |                |                 |       |                     |                      |       |
| Primarily B cell        |                |                 |       |                     |                      |       |
| [BAFF] pg/ml            | 125 ± 66       | 146 ± 107       | 0.04  | 1098 ± 911          | 1052 ± 643           | 0.63  |
| [IL-6] pg/ml            | 10 ± 14        | 8.2 ± 13        | 0.37  | (79) (79)           | (90) (90)            |       |

(Continues)
The hypothesis that OMS does not have a uniform paraneoplastic causation of OMS (Hypothesis 1) arises from several intriguing clues. NB is found in 50% of cases, but the age frequency of OMS with and without NB is identical. NB screening is not always comprehensive; it may create false negatives. Occult NB occasionally can be discovered years after OMS presentation. Some patients with neuroblastoma detected clinically.11 Mechanisms may suggest that occult tumors should be ruled out despite the evidence of infection.21 Of all human tumors, NB has the highest rate of spontaneous regression, and mass-screening program results suggest that there are at least as many children who have tumors undergoing spontaneous regression without detection as there are patients with neuroblastoma detected clinically.11 Mechanisms may be immunologic (humoral or cellular), oncologic (loss of telomerase activity, perturbed epigenetic regulation), or biochemical (deprivation of neurotrophin).11 No consistent infections are found in the non-NB group to suggest a uniform infectious mechanism, and some prodro- 
mal symptoms could result from cytokine neuroinflammation. Patients with and without NB look the same neurologically and respond the same to immunotherapies.12

The hypothesis that OMS does not have a uniform paraneoplastic causation (Hypothesis 2) is also based on supporting data. Virus-
induced diseases of the CNS are widespread, not rare like NB. Of the potential pathogens described in OMS, some are neurotropic59 and/or associated with CSF pleocytosis. APRIL, a proliferation inducing ligand; BAFF, B-cell activating factor; CCL19, C–X–C motif chemokine ligand 19; GFAP, glial fibrillary acidic protein; CXCL13, C–X–C motif chemokine ligand 13; CXCR3, C–X–C motif chemokine receptor 3; IL-1Ra, interleukin-1 receptor antagonist; IL12p40, interleukin-12 p40 subunit.

CXCL12 was measured in plasma. After Bonferroni corrections, there were no statistically significant differences between groups per t-tests.

### Table 2 (Continued)

| Marker       | CSF Tumor | No tumor | Blood/serum Tumor | No tumor | P |
|--------------|-----------|----------|-------------------|----------|---|
| [APRIL] ng/ml| 2.4 ± 1   | 2.4 ± 0.8| 26 ± 37           | 26 ± 42  | 0.98 |
| (66)         | (82)      | (56)     | (58)              |          |    |
| [IL-1Ra] pg/ml| 27 ± 40  | 22 ± 22  | 37 ± 26           | 37 ± 26  | 0.89 |
| (64)         | (66)      | (64)     | (67)              |          |    |
| [IL-16] pg/ml| 33 ± 34  | 32 ± 39  | 237 ± 0.98        | 237 ± 0.98| 0.43 |
| (64)         | (67)      | (68)     | (69)              |          |    |

Data are means ± SD. N values are italicized and in parentheses below the means.

APRIL, a proliferation inducing ligand; BAFF, B-cell activating factor; CCL19, C–X–C motif chemokine ligand 19; GFAP, glial fibrillary acidic protein; CXCL13, C–X–C motif chemokine ligand 13; CXCR3, C–X–C motif chemokine receptor 3; IL-1Ra, interleukin-1 receptor antagonist; IL12p40, interleukin-12 p40 subunit.
FIGURE 4  Comparability of treatment history at evaluation regardless of OMS etiology. Untreated OMS (uOMS), currently treated OMS (cOMS), and previously (not currently) treated (pOMS) at evaluation. (A) Number of patients per treatment category. (B) Box-and-whisker graph of total score (TS) versus treatment category (5–95 percentile whiskers). The tumor group is bolded in the graph for clarity and emphasis (as in (C) and (F)). (C) OMS duration versus treatment status (Tukey whiskers). (D) Type of treatment versus time to treatment. Data are medians with IQR. (E) Comparison of “dramatic response” frequency (per parents) to immunotherapy with IVIg, prednisone, or ACTH. Treatments are not mutually exclusive. Sample sizes are given at the ends of the bars. CST, corticosteroids. (F) TS in patients who had received monotherapy (“Mono”) versus ≥2 conventional agents (“Combo”) (Tukey whiskers)

To help interpret the immunological data in the present study, it should be realized that OMS has been shown to be a neuroinflammatory disorder; the designation “autoimmune,” which has different implications and is not used interchangeably, is suspected, not proven. Advances in research implicating cellular and humoral involvement in OMS over the past two decades have been published in immunology and neuroimmunology journals and may not be known to treating physicians. First, pathologic CSF B-cell expansion in OMS was the evidence-based reason to treat with rituximab. The reduced CD4/CD8 ratio suggests T-cell dysregulation. CSF concentrations of chemokine CXCL13, a B-cell attractant; chemokine CXCL10, an inflammatory chemokine; BAFF, a B-cell activating factor; and cytokine IL-6, a stimulator of antibody production are all elevated in OMS. Second, high
dose steroid and corticotropin reduce the concentrations of inflammatory mediators (not CSF immune cell frequencies)\textsuperscript{34} to varying degrees whether NB was found or not.\textsuperscript{39,40,42–47} Lack of significant differences in these parameters based on apparent OMS etiology is striking.

A great strength of the study was the unprecedented large sample size for pediatric OMS. The multiparameter approach was novel. Patients were systematically evaluated by the same examiners with OMS expertise, and state-of-the-art neuroimmunologic biomarker testing was performed in the same reference laboratory. The multidisciplinary sources of data were also an important asset. Two testable hypotheses were framed along with a synopsis of the supporting literature.

As a limitation, the conclusions are based on the study parameters, and there may be other potentially useful discriminators outside the scope of the study or currently unavailable. Tertiary expert centers for OMS may see more persistent cases than extremely transient ones and may underestimate parainfectious cases. Complete data on types of NB imaging studies or pathogen studies were not available, and this was not a long-term outcome study. Some data are descriptive and associations are reported.

In summary, this large study reveals striking similarities (indistinguishable features) between the “paraneoplastic” and “parainfectious/idiopathic” groups, begging the question of a true etiopathophysiologic dichotomy (two different disorders). Two testable hypotheses are put forward as a stimulus to future research studies. Although cautious interpretation is warranted, the data stress the importance of continued tumor surveillance in the presumed tumor-negative group and increased investigation and documentation of purported pathogens in the presumed parainfectious/idiopathic group. The field seems poised for a breakthrough, pending a blood test or other equally significant refinement for the diagnosis of NB. The data highlight the need for model-building, plans for future research to arrive at a definitive method of settling the etiology issue, and establishing a new language for discussion.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Michael R. Pranzatelli \textsuperscript{ORCID} http://orcid.org/0000-0001-6091-8989

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

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