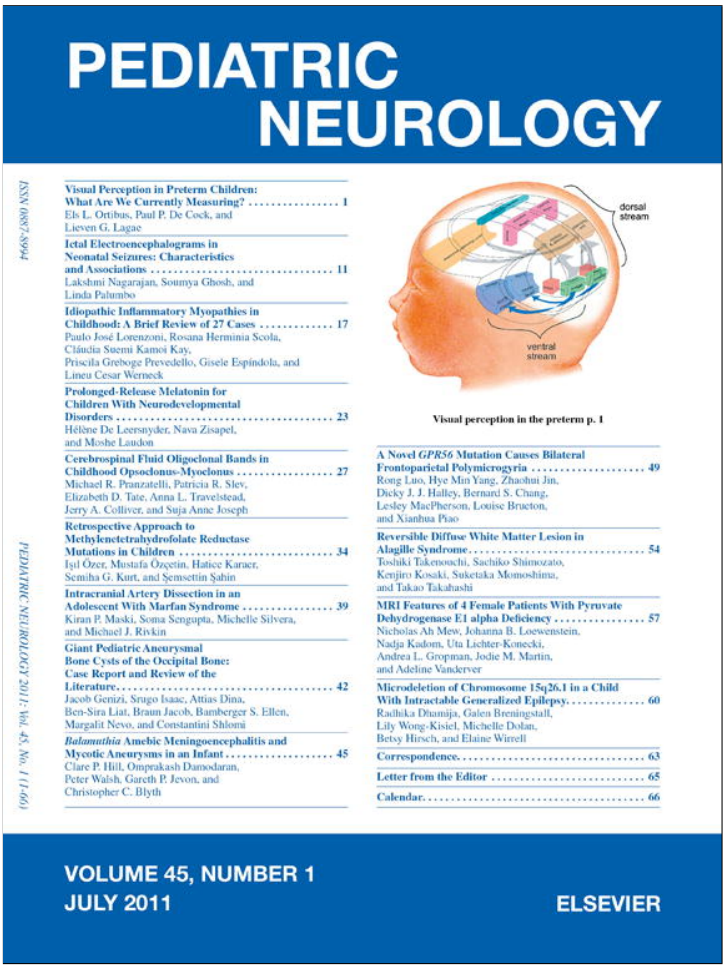

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Original Article

Cerebrospinal Fluid Oligoclonal Bands in Childhood Opsoclonus-Myoclonus

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ABSTRACT

Oligoclonal bands in cerebrospinal fluid reflect local B-cell responses associated with various neuroinflammatory disorders. In opsoclonus-myoclonus syndrome, cerebrospinal fluid B-cell expansion was demonstrated, but no studies of oligoclonal bands are available. In a prospective case-control study of 132 children (103 with opsoclonus-myoclonus, 29 neurologic control subjects), cerebrospinal fluid oligoclonal bands, measured by isoelectric focusing with immunofixation, were observed in 35% with opsoclonus-myoclonus and none of the control subjects, with the highest frequency in severe cases (56%). In oligoclonal band-positive patients, the mean band number was 5 ± 3 S.D. (range, 2-10) and the total severity score was significantly higher than in band-negative patients, whereas the frequency of $CD19^+$ B cells, opsoclonus-myoclonus duration, neuroblastoma detection, and relapse history did not differ. The cerebrospinal fluid immunoglobulin G synthesis rate, immunoglobulin index, and Q albumin were normal. In 17 untreated children receiving adrenocorticotropic hormone, intravenous immunoglobulins, and rituximab, the number of oligoclonal band-positive decreased by 75%, and the mean band count fell by 80%. Oligoclonal band detection adds useful information to neuroimmunologic "staging" in opsoclonus-myoclonus. However, flow cytometry provides a more sensitive measure of B-cell infiltration. Cerebrospinal fluid oligoclonal bands warrant monitoring in long-term follow-up studies of disease-modifying drugs for opsoclonus-myoclonus.

Introduction

Oligoclonal bands, which act as antibodies against a variety of antigens, have long been recognized as a key immunopathologic factor in multiple sclerosis and other neuroinflammatory disorders [1]. Their presence in cerebrospinal fluid but not blood is indicative of an intrathecal humoral immune response [2], and they provide early evidence of B-cell involvement in pathogenesis. The determination of cerebrospinal fluid oligoclonal bands is a standard part of the diagnostic evaluation in those disorders.

Substantial evidence for the role of B-cells was recently observed in pediatric opsoclonus-myoclonus syndrome, an autoimmune paraneoplastic disorder. The frequency of cerebrospinal fluid B-cells is increased several-fold [3], including both T cell-dependent and independent B-cell subsets [4], and systemic treatment with the anti-B-cell monoclonal antibody rituximab (anti-CD20) eliminates B-cell expansion, with clinical benefit [5,6]. However, other than case reports, mostly in adults [7-12], no study of oligoclonal bands in opsoclonus-myoclonus syndrome has been undertaken. Hence, nothing is known about their incidence or utility for risk stratification in pediatric opsoclonus-myoclonus syndrome. Because no surrogate neuroimaging marker, diseased tissue, or animal model for opsoclonus-myoclonus syndrome is available, potential biomarkers of human disease activity need to be evaluated.

This study sought to determine the frequency of oligoclonal band occurrence in opsoclonus-myoclonus cerebrospinal fluid, the relationship of oligoclonal bands to B-cell expansion and key clinical variables, such as relapse, and the effect of immunotherapy. We tested four hypotheses: (1) cerebrospinal fluid oligoclonal bands are related to clinical severity; (2) opsoclonus-myoclonus syndrome with and without neuroblastoma exhibits the same cerebrospinal fluid oligoclonal band frequency, based on our uniform theory of opsoclonus-myoclonus causation [13]; (3) oligoclonal band-positive patients exhibit an increased risk of relapse, whereas oligoclonal band-negative patients manifest a more benign course; and (4) anti-

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B-cell-based combination immunotherapy reduces the frequency of oligoclonal bands.

Study Design and Methods

Opsoclonus-myoclonus population

From 2007-2010, 103 children with opsoclonus-myoclonus syndrome were enrolled in this prospective case-control study through the National Pediatric Myoclonus Center, its website (www.omsusa.org), or physician and family referrals. After a diagnosis of opsoclonus-myoclonus was confirmed by the principal investigator, patients were accepted at any stage of their illness, regardless of treatment status. Parents provided signed, informed consent for their children's participation in this institutional review board-approved study. The mean age of patients was 3.4 ± 2.9 years S.D. (51 boys and 52 girls). The duration of opsoclonus-myoclonus syndrome from onset to evaluation at our center was 1.5 ± 2.9 years (median, 7.4 months). Neuroblastoma (50% at stage I, 44% at stage II, and 6% at stage III) or ganglioneuroblastoma had been surgically removed from all tumor imaging-positive children. Some patients were receiving conventional immunotherapy (alone or in combination), e.g., corticosteroids (33%), intravenous immunoglobulins (70%), or adrenocorticotropic hormone (49%).

Control population

Control subjects included 29 children with noninflammatory disorders and undergoing lumbar puncture for diagnostic testing. Their mean age was 7.2 ± 5.6 years (15 boys and 14 girls). Their age and sex ratios did not differ statistically from those with opsoclonus-myoclonus syndrome. Diagnoses included ataxia ($n = 7$), movement disorders ($n = 8$), factitious or conversion disorders ($n = 3$), headache ($n = 3$), neuroblastoma without opsoclonus-myoclonus syndrome ($n = 3$), and various other disorders ($n = 5$).

Pilot treatment arm

Thirty-one patients undergoing lumbar puncture before and after changes in their medication were eligible as a convenience sample for the longitudinal study. Their interevaluation interval was 7.7 ± 1.2 months (range, 4.6-10.9 months). Seventeen were immunotherapy-naive and received a combination of rituximab, adrenocorticotropic hormone, and intravenous immunoglobulins, using a published treatment schedule [6]. The other 14, an adjunctive group, also received the three agents, but were receiving intravenous immunoglobulin, steroids, adrenocorticotropic hormone, or combinations of these during their first evaluation.

Data collection

Participation in the study entailed a detailed history and neurologic examination, videotaped evaluation sessions, blood tests, and lumbar puncture. The videotapes, which were recorded during the initial visit on the same day as the lumbar puncture, were scored by a trained, blinded rater using the 12-item Opsoclonus-Myoclonus Syndrome Evaluation Scale, in which each item is scored from 0-3 [4]. Severity was categorized as mild for total scores of 0-12, moderate for total scores of 13-24, and severe for total scores of 25-36. The duration of opsoclonus-myoclonus syndrome was considered acute if less than 3 months had passed since its onset, subacute if between 3 months and 1 year had passed, and chronic if more than 1 year had passed. Lumbar punctures were performed under anesthesia, as described previously [3], and cerebrospinal fluid was obtained for cell counts and for the determination of cerebrospinal fluid/serum albumin ratio, immunoglobulin G index, oligoclonal bands, and lymphocyte subset analysis. Blood was drawn at the same time for parallel studies.

Oligoclonal band assay

Samples were frozen and shipped on dry ice to the ARUP Laboratories (Salt Lake City, UT) for the oligoclonal band profile (test number 0080440). Isoelectric focusing and immunofixation were performed on paired sera (1 mL) and unconcentrated cerebrospinal fluid (0.75 mL), which were diluted to equivalent concentrations of immunoglobulin G and run side by side. The Hydragel 9 Cerebrospinal Fluid Isofocusing Kit, designed for the qualitative detection and identification of oligoclonal bands, and the semiautomated Hydrasys System (boith from Sebia, Norcross, GA) were used. The assay is based on the high-resolution electrophoretic separation of immunoglobulin G, because of the different charges or isoelectric points on agarose gel, and on immunofixation with enzyme-labeled antisera against human immunoglobulin G (package insert, publication number 2005/04, Sebia). Immunofixation allows for visualization and increases specificity, because the banding is specific for immunoglobulin G [14]. Two or more bands in the cerebrospinal fluid are considered a positive result [15,16].

As a test of reproducibility, oligoclonal bands were measured on a second cerebrospinal fluid and serum aliquot from the same sample collection in eight oligoclonal band-positive patients. The grouped data were compared statistically, using a paired t test. The mean numbers of oligoclonal bands with standard deviations were 5.8 ± 4.9 in the first samples and 5.8 ± 3.9 in the second samples, which were not significantly different.

Immunoglobulin G/albumin indexes

Immunoglobulin and albumin concentrations were measured in both serum and cerebrospinal fluid, using the BN II Nephelometer (Siemens Dade Behring, Schwalbach, Germany). Immunoglobulin G and albumin indices were calculated as previously described, to determine the permeability status of the blood-brain barrier [17]. immunoglobulin G synthesis rate was calculated by the ARUP Laboratories, using a formula requiring five values: cerebrospinal fluid immunoglobulin G − serum immunoglobulin G/369; cerebrospinal fluid albumin − serum albumin/230 (value A); serum immunoglobulin G × 0.43 (value B); serum albumin (value C); and value A × value B ÷ value C. Value 1 − value 2 × 5 equaled the immunoglobulin G synthesis rate. Q albumin is standard nomenclature for the cerebrospinal fluid/serum albumin quotient.

Flow cytometry

Cerebrospinal fluid and blood immunophenotyping was performed by four-color, dual-laser flow cytometry, as detailed previously [3]. A panel of directly conjugated monoclonal antibodies to CD27, CD19, CD3, immunoglobulin D, and immunoglobulin G isotypes was used. Total B cells were defined as $CD19^+CD3^-$, memory B cells were defined as $CD19^+CD27^+$, and naive B cells were defined as $CD19^+CD27^-$ immunoglobulin D^+. The purity of the lympho-monocyte gate was checked by double staining with CD45, a pan-leukocyte marker, and CD14, a monocyte marker. Multiple measurements were performed to maintain quality control [3]. The net "percentage of lymphocytes" refers to the percentage of cells positive within the gate defined for the specific combination of antibodies.

Statistical analysis

Demographic data were evaluated by descriptive statistics. Laboratory data were analyzed as means ± standard deviation (cross-sectional data) or ± standard error of the mean (longitudinal data) by two-tailed t tests or analysis of variance with the Tukey multiple comparison test. If variances were unequal, the Kruskal-Wallis test was used instead of analysis of variance. For data analyzed as frequencies, the χ^2 test or Fisher exact test was used to compare categorical variables. The primary group comparisons were between untreated patients with opsoclonus-myoclonus syndrome and control subjects, and between untreated and treated patients with opsoclonus-myoclonus syndrome. Pretreatment and posttreatment data were analyzed by paired t tests. For correlation analyses, Pearson correlations were used. In secondary analyses, logistic regressions were performed on the longitudinal dataset, using the Statistical Analysis System, Cary, NC.

Results

Cross-sectional study

The inspection of a gel (Fig 1) reveals various cerebrospinal fluid bands from patients with opsoclonus-myoclonus syndrome that

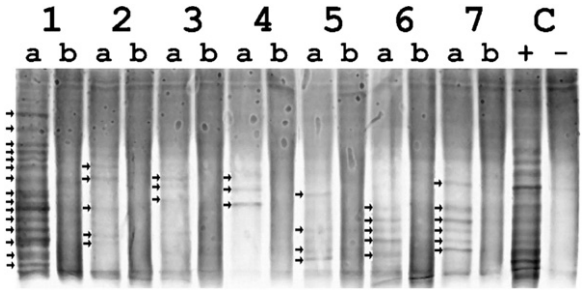

Figure 1. A gel was produced for photographic documentation of seven oligoclonal band-positive patients with opsoclonus-myoclonus syndrome. Blots were interpreted manually, comparing cerebrospinal fluid (lane a) and serum (lane b) oligoclonal bands. From left to right, the numbers of bands in cerebrospinal fluid lanes (arrows) not evident in serum lanes comprised 15, 5, 3, 3, 4, 5, and 6. Patients 1-4 were part of the tumor group. Patients 5-7 were part of the group of patients in whom no tumor was evident. A known positive control (two or more bands) and negative control (no bands) were included on the right (lane C, + and −). All patients were male, and both groups were age-matched: mean age, 2.2 years for the tumor group, and 2.5 years for the group in which no tumor was evident. Patient 2 was scored as acute. Patients 1 and 7 were scored as chronic. The rest were scored as subacute. Patient 6 was immunotherapy-naive.

are not evident in serum (alternate lanes), and varying between patients in frequency and position. The groups with and without neuroblastoma could not be visually differentiated in this gel or in other gels (not shown).

Two or more cerebrospinal fluid oligoclonal bands were detected in 35% of patients with opsoclonus-myoclonus syndrome and none of the control subjects (Table 1). The frequency of detection was highest in severe cases (56%). The mean number of bands was comparably higher in severe (Fig 2), untreated, and acute opsoclonus-myoclonus categories. Moreover, 12 (12%) of all patients with opsoclonus-myoclonus syndrome, but no control subjects, had just one oligoclonal band. No significant effects of opsoclonus-myoclonus duration, tumor detection, or treatment with conventional immunotherapy were evident. The percentage of total B cells in cerebrospinal fluid trended the same way, but also correlated with duration of opsoclonus-myoclonus syndrome.

None of the children demonstrated an abnormal rate of cerebrospinal fluid immunoglobulin G synthesis or immunoglobulin G index. One patient exhibited an elevated albumin index of 11.5 (normal range, <9), but was oligoclonal band-negative. She had manifested opsoclonus-myoclonus syndrome for 16 years, and was not in remission at the time of her visit (total score, 24).

Oligoclonal band-positive vs band-negative patients

The dataset was divided into oligoclonal band-positive and band-negative groups (Table 2). The groups were well balanced in terms of patient age, sex, and onset and duration of opsoclonus-myoclonus syndrome. The opsoclonus-myoclonus mean severity score was 46% higher in the oligoclonal band-positive group, in which 81% of patients were scored as moderate or severe, compared with 40% in the oligoclonal band-negative group. The oligoclonal band-positive group did not exhibit a higher frequency of cerebrospinal fluid B cells, which was above normal in both groups. The percentages of children with neuroblastoma and tumor stages were not significantly different between groups.

Table 1. Cerebrospinal fluid oligoclonal bands in cross-sectional dataset in relation to possible clinical factors

	n	No. Positive (%)	*P*	No. of Bands in Mean ± S.D.	OCB-Positive (Range)	*P*
Group						
Controls	28	0 (0)				
OMS	103	36 (35)		5.3 ± 2.6	(2-10)	
OMS duration			0.93			0.24
Acute	30	11 (37)		6.2 ± 2.5	(2-9)	
Subacute	39	14 (36)		4.5 ± 2.4	(2-10)	
Chronic	34	11 (32)		5.4 ± 2.7	(2-10)	
OMS etiology			0.07			0.87
Tumor	56	15 (27)		5.3 ± 2.4	(3-10)	
"No tumor"	47	21 (45)		5.2 ± 2.7	(2-10)	
OMS severity			0.0004*			0.31
Mild	47	7 (15)		4.3 ± 2.6	(2-9)	
Moderate	38	19 (50)		5.1 ± 2.7	(2-10)	
Severe	18	10 (56)		6.2 ± 2.0	(3-8)	
Treatment			0.60			0.16
Untreated	19	8 (42)		6.4 ± 2.3	(2-9)	
Treated	84	28 (33)		4.9 ± 2.6	(2-10)	
Relapse history			0.22			0.44
Nonrelapser	48	20 (42)		5.6 ± 2.7	(2-10)	
Relapser	55	16 (29)		4.9 ± 2.4	(3-10)	

Abbreviations:
OCB = Oligoclonal band
OMS = Opsoclonus-myoclonus syndrome
S.D. = Standard deviation

Percentages represent the proportion of band-positive patients in each category. No significant differences were evident in terms of male/female ratio, mean age at onset, mean age at evaluation, or mean duration between groups (data not shown). For continuous variables (numbers of bands), two-group comparisons were performed using two-tailed *t* tests. Because control subjects exhibited no oligoclonal bands, the test could not be performed on them. Three-group comparisons of means were performed using analysis of variance. The numbers of patients with OCB were compared according to χ^2 or Fisher exact tests. No results of χ^2 tests could be computed for comparisons of band frequency between control subjects and patients with OMS, because no control subjects manifested any bands.

* Statistically significant difference.

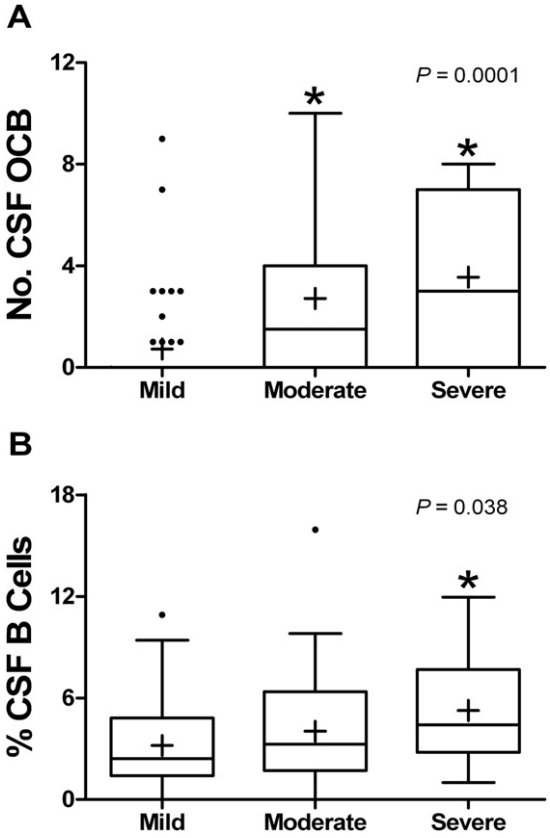

Figure 2. Relationship of severity category in opsoclonus-myoclonus syndrome to (A) the number of cerebrospinal fluid oligoclonal bands (CSF OCB) and (B) the frequency of cerebrospinal fluid B cells in the entire cross-sectional dataset, regardless of duration and severity of opsoclonus-myoclonus syndrome or previous treatment. Box-and-whisker graphs depict the means (+), medians (horizontal lines within boxes), 75th-25th percentile interquartile ranges (upper and lower edges of boxes, respectively), and Tukey error bars (greatest and least values, excluding outliers). Statistical outliers (i.e., more than 1.5 times the upper quartile) are depicted as dots. The statistical analysis of data in B (equal variances) was performed using analysis of variance, and in A (unequal variances) using the Kruskal-Wallis test, although analysis of variance produced the same level of significance. Asterisks indicate significant differences compared with the mild category ($P < 0.05$, Tukey test).

Correlations

In the total cross-sectional dataset, a significant correlation was evident between total score and the number of cerebrospinal fluid oligoclonal bands ($r = 0.38$, $P < 0.0001$). However, many patients without bands demonstrated high severity scores and cerebrospinal fluid B cell expansion, and when they were excluded, the correlation was no longer significant. The total score and the frequency of cerebrospinal fluid B cells were also correlated ($r = 0.29$, $P = 0.0033$), whereas oligoclonal bands and total cerebrospinal fluid B cells were not correlated ($r = 0.13$, $P = 0.19$). No significant correlations were evident between oligoclonal band counts and percentages of cerebrospinal fluid memory or naive B cells (the same finding was true for blood), duration or age at onset of opsoclonus-myoclonus syndrome, patient age, or conventional immunotherapy (data not shown). In the oligoclonal band-positive

Table 2. Phenotypic comparison of oligoclonal band-positive and oligoclonal band-negative patients

	OCB-Positive ($n = 36$)	OCB-Negative ($n = 67$)	P
Age (yr)	3.3 ± 2.2	3.5 ± 3.3	0.69
Male/female	20:16	31:36	0.41
OMS onset (yr)	2.0 ± 1.6	1.9 ± 1.2	0.57
OMS duration (yr)	1.2 ± 1.9	1.6 ± 3.3	0.50
Tumor	15 (42%)	41 (61%)	0.07
Stage I	7	10	0.37
Stage II	5	14	1.0
Stage III	0	1	
Ganglioneuroblastoma	0	7	1.0
Unknown	3	9	1.0
OMS severity (total score)	19.1 ± 8.0	13.1 ± 8.4	<0.0007*
OMS severity category			0.0004†
Mild (%)	7 (19%)	40 (60%)	
Moderate (%)	19 (53%)	19 (28%)	
Severe (%)	10 (28%)	8 (12%)	
Relapse history			
Nonrelapser	20 (56%)	28 (42%)	0.22
Relapser	16 (44%)	39 (58%)	0.44
Cerebrospinal fluid leukocytes	3.1 ± 2.6	2.7 ± 3.0	0.58
Cerebrospinal fluid B cells (%)‡	4.5 ± 3.4	3.5 ± 2.6	0.10
Cerebrospinal fluid memory B cells (%)	2.3 ± 1.2	2.6 ± 2.2	0.57
Blood B cells (%)	23.1 ± 8.4	24.2 ± 10.8	0.62
Cerebrospinal fluid IgG	0.9 ± 0.8	1.2 ± 1.7	0.50
Serum IgG	953 ± 379	835 ± 337	0.20
IgG index	0.46 ± 0.24	0.40 ± 0.27	0.37
IgG synthesis rate	<3	<3	
Q albumin	2.2 ± 0.65	2.8 ± 1.7	0.08

Abbreviations:
IgG = Immunoglobulin G
OCB = Oligoclonal band
OMS = Opsoclonus-myoclonus syndrome
S.D. = Standard deviation

Values represent means ± S.D. Percentages are presented within parentheses after frequencies. The number of patients for cerebrospinal fluid memory B cell % in each respective group was 16 and 36; for the IgG index, 25 and 34; and for Q albumin, 27 and 38. The cerebrospinal fluid leukocyte count is expressed as cells/mm^3, and the rate of IgG synthesis is expressed as mg/day.

Neuroblastomas were staged using the International Neuroblastoma Staging System. Ganglioneuroblastoma was not staged.

* Significant unpaired t test.

† Significant χ^2 test or Fisher exact test.

‡ Mean frequency of B cells in control subjects was <1%.

opsoclonus-myoclonus group, the number of oligoclonal bands did not correlate but perhaps trended ($r = 0.32$, $P = 0.06$) with the concentration of cerebrospinal fluid immunoglobulin G. In patients with opsoclonus-myoclonus syndrome treated without chemotherapy ($n = 47$), oligoclonal bands again correlated with total score ($r = 0.47$, $P < 0.0006$), as they did when all treatments were included ($n = 63$, $r = 0.48$, $P < 0.0001$).

Longitudinal study: Treatment effects

In the 17 immunotherapy-naive patients with opsoclonus-myoclonus syndrome treated prospectively with a combination of adrenocorticotropic hormone, intravenous immunoglobulin, and rituximab (Fig 3), the number of positive cerebrospinal fluid oligoclonal bands fell significantly, from 5.0 ± 1 pretreatment to 1.0 ± 0.8 posttreatment. The number of patients with ≥2 bands decreased from 8 to 2 ($P = 0.057$, Fisher exact test), and the number of patients with ≥1 band decreased from 10 to 3 ($P = 0.032$). The frequency of cerebrospinal fluid B cells and total scores were also significantly reduced.

In the entire group of 31 children (data not shown), the number of oligoclonal bands was reduced significantly, from 2.3 ± 0.5 pretreatment to 0.6 ± 0.2 posttreatment ($P = 0.0022$). The percentage of oligoclonal band-positive patients decreased from 13 to 4 ($P = 0.021$). Three patients remained band-positive (≥2 oligoclonal bands), and five retained only one oligoclonal band. The cerebrospinal fluid B-cell percentage declined from 5.7% ± 0.6% to 0.3% ± 0.1% (−95%, $P < 0.0001$), and total scores decreased from 21.0 ± 1.3 to 7.4 ± 0.7 (−65%, $P < 0.0001$).

When the 13 children with pretreatment oligoclonal band positivity were analyzed separately, the posttreatment band frequency was reduced by 77%, to a frequency of 23%. The mean number of oligoclonal bands decreased significantly from 5.1 ± 0.7 to 0.92 ± 0.5, the percentage of cerebrospinal fluid B cells decreased from 6.7% ± 1.0% to 0.3% ± 0.1%, and total scores decreased from 21.7 ± 1.9 to 7.2 ± 1.1. In eight patients, the cerebrospinal fluid oligoclonal bands decreased to 0.

The pretreatment oligoclonal band-negative subgroup of 18 was also analyzed separately. Seventeen (94%) children remained band-negative during the follow-up period. The remaining patient became oligoclonal band-positive.

Three patients posttreatment developed elevated cerebrospinal fluid immunoglobulin G synthesis rates of 8.1, 9.8, and 32.9 mg/day (normal range, 0-8). They had been treated with rituximab 6, 16, 10 months earlier. None had relapsed, and each was in the mild range of motor severity (total scores of 6, 5, and 4). The patient with the highest rate demonstrated an elevated immunoglobulin G index of 0.87 (normal range, 0.28-0.66). The other two also demonstrated the highest albumin indexes, at 14.6 and 16.8 (normal range, 0-9). The child with the highest rate of immunoglobulin G synthesis exhibited a normal value on cerebrospinal fluid testing before and after that sample. The two highest had positive oligoclonal bands (17 and 7 bands).

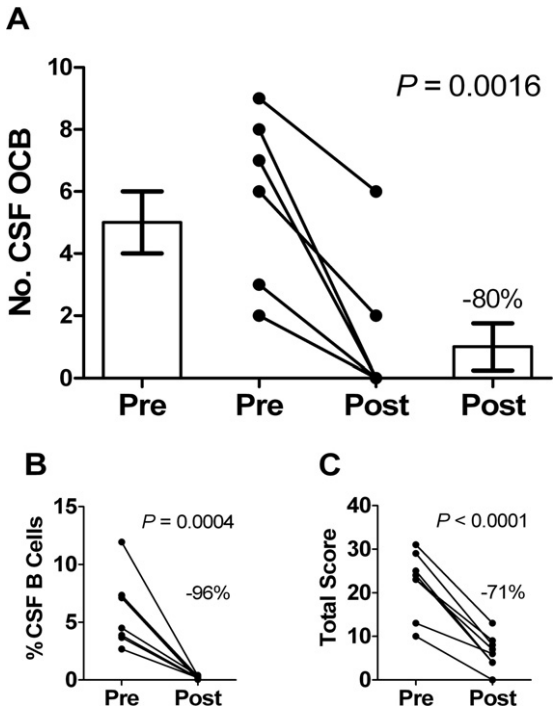

Figure 3. Effects of combination treatment with rituximab, adrenocorticotropic hormone, and intravenous immunoglobulins on (A) cerebrospinal fluid oligoclonal band (CSF OCB) number, (B) percentages of B cells, and (C) total scores in 8 previously untreated patients. Statistical analysis, using paired t tests, is depicted for children with ≥2 oligoclonal bands during their first evaluation. The results were similar when those with one band were also included, i.e., 2.5 ± 3.1 bands pretreatment, and 0.5 ± 1.5 bands posttreatment (−79%, $P = 0.0031$) (n = 17). Means are shown with S.E.M.

Predictions of relapse

A secondary statistical analysis of relapse was performed on the dataset of 31 treated patients. Of the 13 initially oligoclonal band-positive patients, four (31%) relapsed; of the 18 oligoclonal band-negative patients, seven (39%) relapsed. Oligoclonal bands persisted in two patients who did not relapse, with serial band counts of 10, 7, and 7 in one patient, and 6, 2, and 3 in the other. Longitudinal comparisons of oligoclonal bands, relapses, and total scores did not indicate a consistent pattern (Fig 4).

A correlation analysis was performed on the combined pretreatment and posttreatment data. Pretreatment total score ($r = 0.48$, $P = 0.0005$), change in total score ($r = 0.48$, $P = 0.0084$), and posttreatment total score ($r = 0.30$, $P = 0.037$) correlated with relapse, but not with the number of relapses. The pretreatment number of oligoclonal bands correlated with the change in total score ($r = 0.33$, $P = 0.024$). To test the predictive value of oligoclonal bands in relapse, a logistic regression was performed with the same dataset. Pretreatment oligoclonal band number did not predict relapse ($P = 0.23$).

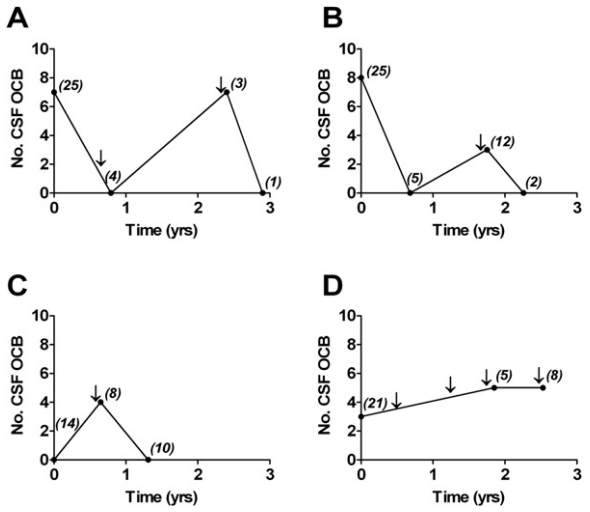

Figure 4. Longitudinal course of four relapsing patients who had undergone three or more evaluations of cerebrospinal fluid. Numbers in parentheses indicate the patient's severity score, and arrows indicate relapses. Changes in treatments are described. (A) A 14-month-old child with opsoclonus-myoclonus syndrome, untreated at initial visit, began receiving adrenocorticotropic hormone, intravenous immunoglobulins, and rituximab. A relapse occurred before the second evaluation, which was treated with a single additional infusion of rituximab and an increased dose of adrenocorticotropic hormone. Another relapse occurred after 2 months off adrenocorticotropic hormone, prompting a third visit that revealed rebounded oligoclonal bands. Therefore, adrenocorticotropic hormone was reinitiated, and three cycles of intravenous cyclophosphamide (1 g/m^2) were added. (B) An 18-month-old child had been receiving steroids, intravenous immunoglobulins, and rituximab, and adrenocorticotropic hormone was substituted for the steroids. By the third visit, after the patient had been off adrenocorticotropic hormone for 5.5 weeks, a relapse had occurred. Intravenous immunoglobulins were continued, pulse dose dexamethasone (20 mg/m^2/day × 4 consecutive days/month) and rituximab were administered, and the child was later evaluated while receiving dexamethasone and intravenous immunoglobulins alone. (C) A 17-month-old child treated with adrenocorticotropic hormone, intravenous immunoglobulins, and rituximab relapsed by the second visit, and three cycles of cyclophosphamide (0.75 g/m^2) were administered, with an increased dose of adrenocorticotropic hormone. On the third visit, the patient was receiving adrenocorticotropic hormone and intravenous immunoglobulins. (D) A 34-month-old child who had undergone several previous relapses was first evaluated while receiving adrenocorticotropic hormone, and additional immunotherapy was recommended but not implemented. Relapses occurred before the second visit, after which the dose of adrenocorticotropic hormone was increased and three cycles of cyclophosphamide (0.75 g/m^2) and intravenous immunoglobulins were added. Another relapse occurred near the time of the third visit. CSF OCB = cerebrospinal fluid oligoclonal band.

Discussion

The finding of clonal intrathecal immunoglobulin G of restricted diversity in childhood opsoclonus-myoclonus syndrome strengthens the humoral hypothesis of its pathogenesis, which is based on reports of various immunoglobulin G autoantibodies in cerebrospinal fluid [18-21] and of cerebrospinal fluid B-cell expansion [3,4]. The cerebrospinal fluid restriction of oligoclonal bands is supported by the absence of an elevated albumin index (except in one patient) that might otherwise suggest a "leaky" blood-brain barrier. The antigenic substrate for immunoglobulins is unknown, and we are performing studies to determine whether cross-reactivity against human brain or neuroblastoma exists. Recently, elevated cerebrospinal fluid levels of B-Cell Activating Factor, which stimulates immunoglobulin production, were also observed in opsoclonus-myoclonus syndrome [22].

Cerebrospinal fluid oligoclonal bands are detected in various neuroinflammatory disorders, such as multiple sclerosis, systemic lupus erythematosus, neurosarcoidosis, and Behcet's disease [2,23], as well as in certain neuroinfectious disorders, such as neuroborreliosis, neurosyphilis, and aseptic meningitis [24,25]. In 10 adults with paraneoplastic cerebellar degeneration and encephalomyelitis/sensory neuropathy [26], a range of 2-16 cerebrospinal fluid oligoclonal bands were detected, and oligoclonal bands may also be directed toward specific onco-neural antigens [26,27]. In comparison with multiple sclerosis, in which more than 95% of patients manifest cerebrospinal fluid oligoclonal bands [28], the frequency and number of oligoclonal bands in opsoclonus-myoclonus syndrome were much lower, and more similar to those in neuromyelitis optica, in which 23% of patients are oligoclonal band-positive [29], but higher than the 10% observed in acute disseminated encephalomyelitis [30]. Similarly, the cerebrospinal fluid immunoglobulin G index was elevated in 70-90% of patients with multiple sclerosis (rarely in oligoclonal band-negative cases) [28], but in opsoclonus-myoclonus syndrome, the index was usually normal. The correlation of elevated cerebrospinal fluid leukocytes and oligoclonal bands observed in multiple sclerosis [31,32] was not evident in children with opsoclonus-myoclonus syndrome, in which cerebrospinal fluid leukocytosis is usually minimal or absent.

Although a single immunoglobulin G band in cerebrospinal fluid that is absent from serum is indicative of intrathecal synthesis in theory, two or more bands are interpreted as a dependable indication in practice. In opsoclonus-myoclonus syndrome, given the sensitivity of the detection method, perhaps even one oligoclonal band (sometimes referred to as "borderline") should be considered positive, insofar as none were evident in pediatric control subjects. This finding, of course, begs the question of *oligo*clonal. According to the reference laboratory, the requirement for two bands provides greater protection from a false-positive result. Furthermore, band stringency varies between commercial laboratories. A few require ≥4 bands to be positive, based on experience with multiple sclerosis.

The source of oligoclonal bands in the cerebrospinal fluid of patients with multiple sclerosis is thought to be the plasma cell (CD19$^-$), which finds a long-term survival niche in the central nervous system [1]. Oligoclonal bands are notorious for their persistence for years in the cerebrospinal fluid of patients with multiple sclerosis [33], irrespective of the course or therapy of the disease [28], as is also the case for central nervous system infections [24]. In our study, cerebrospinal fluid oligoclonal bands were present many years after the onset of opsoclonus-myoclonus syndrome, despite conventional immunotherapy, in children with persistent opsoclonus-myoclonus syndrome. The correlation of oligoclonal bands with the frequency of cerebrospinal fluid plasma cells in opsoclonus-myoclonus syndrome remains to be tested.

The detection of cerebrospinal fluid oligoclonal bands is not straightforward, given the differences in yield depending on the methodology used by the laboratory. Isoelectric focusing combined with immunofixation comprises the gold standard assay for the detection of oligoclonal bands [2,28,34]. Previously, no cerebrospinal fluid oligoclonal bands were evident in 28 of our other pediatric patients with opsoclonus-myoclonus syndrome, using agarose gel electrophoresis without isolectric focusing (unpublished data).

In addition to oligoclonal bands, a significant component of cerebrospinal fluid immunoglobulin is also polyclonal, potentially allowing for the recognition of antigens directed at the central nervous system and other sites [35]. Polyclonal immunoglobulin may reflect the continued activation of memory B cells and plasma cells that have merely accessed the central nervous system [35]. Cerebrospinal fluid memory B cells had increased in our patients. Moreover, immunoglobulin M oligoclonal bands may have been present, but an assay for immunoglobulin M oligoclonal bands is not routinely available at commercial laboratories.

The presence or absence of oligoclonal bands in multiple sclerosis is used as a classification factor to study prognostic and immunologic features. In a recent study of 415 patients, the detection of oligoclonal bands almost doubled the risk for a second attack [36], but did not hasten the development of disability [36,37]. In this study of opsoclonus-myoclonus syndrome, oligoclonal bands did not correlate with previous or subsequent relapses, although a much larger dataset will be required for definitive results.

Based on multiple sclerosis, a correlation between oligoclonal bands and cerebrospinal fluid immunoglobulin G concentration, immunoglobulin G index, or B cells might be expected. However, the findings for opsoclonus-myoclonus syndrome were quite different, and the reasons for this difference are not clear. In multiple sclerosis, the activation of immunoglobulin genes seems to occur within plasma cells in the meninges, suggesting that the origin of immunoglobulin-producing B cells in cerebrospinal fluid could be meningeal [38], but no data are available for opsoclonus-myoclonus syndrome. Perhaps cerebrospinal fluid B cells secrete a different isotype, or else cerebrospinal fluid plasmablasts and plasma cells, which we are beginning to measure, may offer better prospects for correlation. Certainly, to analyze 100 untreated children with opsoclonus-myoclonus syndrome, rather than a heterogeneous population, would be more valid for such correlations, although those 100 untreated children would be difficult to recruit in such a rare disorder.

The frequency and number of oligoclonal bands in children with or without apparent neuroblastoma, which is evident in about 50% of pediatric patients with opsoclonus-myoclonus syndrome [3], did not differ significantly. However, the etiology of opsoclonus-myoclonus syndrome in cases where no tumor is detected remains controversial. Spontaneous tumor regression, missed tumors, and viral causation have been proposed [13].

The decrease in number of oligoclonal band-positive patients after immunotherapy with rituximab, adrenocorticotropic hormone, and intravenous immunoglobulins was encouraging, but must be interpreted with caution, given our limited sample size. Whether the combination of agents or rituximab made the difference remains unclear, because our cross-sectional study indicated that conventional agents alone do not reduce the oligoclonal bands in opsoclonus-myoclonus syndrome. If cerebrospinal fluid oligoclonal bands in opsoclonus-myoclonus syndrome are derived from long-lived plasma cells, rituximab would not be expected to eradicate them, because plasma cells lack the CD20 surface antigen to which it binds. In contrast, pools of plasmablasts and short-term plasma cells, which constitute other potential sources of autoantibodies, are indirectly vulnerable to rituximab through the elimination of their B-cell precursors.

In conclusion, this study presents new data on the intrathecal humoral immune response in opsoclonus-myoclonus syndrome and its relationship to cerebrospinal fluid B-cell expansion, clinical variables, and treatment. One take-home message for the clinician states that oligoclonal band testing should be performed by a laboratory that uses isoelectric focusing with immunofixation. This test dichotomizes opsoclonus-myoclonus syndrome into band-positive and band-negative subgroups, the clinical and immunologic significance of which remain to be demonstrated. Because an absence of bands does not exclude cerebrospinal fluid B-cell expansion, oligoclonal bands are not a stand-alone biomarker in opsoclonus-myoclonus syndrome. Therefore, we are studying whether they possess greater utility in a biomarker panel. Finally, we are at the preliminary stages of understanding humoral responses in pediatric opsoclonus-myoclonus syndrome compared with more common autoimmune disorders, such as multiple sclerosis, and larger studies of treatment-naive and prospectively treated children will be necessary to confirm our results.

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