

Evidence of Cellular Immune Activation in Children With Opsoclonus-Myoclonus: Cerebrospinal Fluid Neopterin

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ABSTRACT

To evaluate cellular immune activation in opsoclonus-myoclonus syndrome, we measured the inflammatory marker neopterin in the cerebrospinal fluid of 16 children with opsoclonus-myoclonus and neuroblastoma, 24 children with opsoclonus-myoclonus but no tumor, and 19 age-matched controls. The mean concentration in opsoclonus-myoclonus was 2.3-fold higher than in controls ($P = .008$). Neopterin was greatly elevated in four of the most neurologically severe cases, up to 8.3-fold above the highest control level. Thirteen of the 40 children with opsoclonus-myoclonus but no controls had a neopterin concentration > 2 SD above the control mean ($P = .005$). In this high neopterin subgroup, neurologic severity was significantly greater and the duration of neurologic symptoms was less. In 16 children re-examined on immunotherapy, including adrenocorticotropic hormone (ACTH) combination therapy, treatment was associated with a significant reduction in both neopterin and neurologic severity. Neopterin did not differ significantly between the tumor and non-tumor opsoclonus-myoclonus etiologies. No abnormalities of tetrahydrobiopterin were found. Although cerebrospinal fluid neopterin lacked the sensitivity to be a biomarker of disease activity in opsoclonus-myoclonus, elevated concentrations do support a role for T-cell activation and cell-mediated immunity in its pathophysiology. (*J Child Neurol* 2004;19:919–924).

Childhood-onset opsoclonus-myoclonus syndrome is a neuropsychiatric disorder¹ that is typically associated with occult neuroblastoma.² Even when a tumor cannot be found, the chance that one underwent spontaneous regression is high.³ The principal theory of opsoclonus-myoclonus pathogenesis is autoimmune cross-reactivity between brain and tumor antigens.⁴ Although there is evidence for humoral and

cellular mechanisms in adult paraneoplastic syndromes, less is known about the nature and source of immune system dysfunction in the childhood-onset disorder, and possible cellular mechanisms have been particularly neglected.⁵

Neopterin is an indicator of cellular immune activation.^{6–8} It is an unconjugated pteridine that is biosynthetically derived from guanosine triphosphate via 7,8-dihydroneopterin triphosphate. Dephosphorylation leads to the production of 7,8-dihydroneopterin, the form in which more than 70% of cerebrospinal fluid neopterin exists.⁹ The concentration of neopterin in cerebrospinal fluid has been shown to be a more reliable marker of inflammation involving the central nervous system than serum levels.¹⁰ Cerebrospinal fluid neopterin has been measured in a wide variety of autoimmune and infectious diseases in which T cells and macrophages are involved^{11,12} but not previously in opsoclonus-myoclonus.

We now report on cerebrospinal fluid neopterin in a series of children at various stages in the course of their opsoclonus-myoclonus and the response to immunotherapy, with detail given to particularly severe index cases found to have the highest levels of neopterin. For this study, we

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enlisted opsoclonus-myoclonus children with and without neuroblastoma and pediatric neurologic controls. Tetrahydrobiopterin (BH₄) also was evaluated as an internal control.

METHODS

Subjects

Forty children with opsoclonus-myoclonus were enrolled through the National Pediatric Myoclonus Center after parents signed informed consent for this institutionally approved study. Forty-three percent were male. Neuroblastoma, which was found in 16 patients, had been resected prior to the study, and 56% of those had also received tumor chemotherapy. The mean age of children with neuroblastoma (3.38 ± 0.51 years) did not differ significantly from other opsoclonus-myoclonus (4.60 ± 0.77 years). The mean time from onset of opsoclonus-myoclonus to lumbar puncture (syndrome duration) was 2.31 ± 0.47 years (range 0.25–14.4 years). Twenty-three percent of all children with opsoclonus-myoclonus were not receiving immunotherapy at the time of the first lumbar puncture, but 85% had received treatment in the past.

Controls were 19 children with neurologic but nonimmunologic disorders, including chronic headaches, movement disorders, or epilepsy.

Forty-two percent of the subjects were male. The mean age for controls (5.63 ± 0.96 years) was not statistically different than for the children with opsoclonus-myoclonus (4.12 ± 0.51 years).

Lumbar Puncture

Lumbar puncture was performed under propofol intravenous anesthesia to reduce the risk of contamination of cerebrospinal fluid with blood and lessen the effects on the immune system, as well as for compassionate care.¹³ The first 3 mL of fluid was sent for routine studies, and then 0.75 mL of the next 1 mL was aliquoted to an Eppendorf tube containing dithioerythritol and diethylenetriaminepentaacetic acid for pterins.¹⁴ Seventeen children underwent a second lumbar puncture after various treatments. Only three children with opsoclonus-myoclonus had elevated cerebrospinal fluid white blood cell counts, and protein and glucose levels were normal.

Scoring of Videotapes

A trained observer blinded to treatment status rated motor impairment using the Opsoclonus-Myoclonus Evaluation Scale, which we devised and validated.¹⁵ As an index of increasing neurologic severity, items of the 12-part scale were rated from 0 to 3, and total score was calculated as the sum of subscores. A total score of 36 indicates maximum abnormality. Two patients were not videotaped.

Treatment

After the initial lumbar puncture, 17 patients were treated with various immunotherapies, alone or in combination. When adrenocorticotropic hormone (ACTH) was used, it was injected intramuscularly at high doses, as described previously.¹⁶ Intravenous immunoglobulin was begun at 2 g/kg divided over 2 days and maintained at 1 g/kg once monthly. Cyclophosphamide was administered intravenously at 750 mg/m² once monthly for six cycles. Most children received more than one agent because in the experience of the

National Pediatric Myoclonus Center, combination therapy is associated with fewer relapses and a better neurologic outcome.

Assays

The neopterin-tetrahydrobiopterin sample was frozen and shipped on dry ice to the Baylor Institute of Metabolic Research. Both neopterin (dihydroneopterin) and 5,6,7,8-tetrahydrobiopterin were separated by reverse-phase high-performance liquid chromatography and measured by in-series electrochemical and fluorescence detection, as described previously.¹⁴

Statistical Analysis

All statistical analysis used the *Statistical Analysis System* (SAS) (SAS Institute Inc., Cary, NC), a computer software program for statistical analysis.¹⁷ Data were analyzed as means by *t*-tests or analysis of variance (ANOVA) with Duncan's Multiple Range Test. Correlation analysis was performed to evaluate a possible relation between biochemical and clinical variables. Repeat cerebrospinal fluid measurements were analyzed by paired *t*-tests. Numbers of subjects were analyzed by chi-square or Fisher exact test. A significance level of $P < .05$ was used for all statistical analysis.

RESULTS

Cross-sectional Study

Analysis of All Opsoclonus-Myoclonus

Cerebrospinal fluid neopterin was higher in the opsoclonus-myoclonus group, whether analyzed as group means or as the number of individuals with elevations. Mean neopterin in the opsoclonus-myoclonus group (Figure 1) was 131% greater than in controls ($P = .008$). Four children exhibited striking elevations in neopterin, up to 8.3-fold above the highest control value. There were no significant differences in tetrahydrobiopterin concentrations.

Neopterin and tetrahydrobiopterin concentrations did not differ significantly in paraneoplastic opsoclonus-myoclonus compared with opsoclonus-myoclonus without a tumor (Table 1). Although neopterin was somewhat higher in females with opsoclonus-myoclonus, there was no significant gender effect in controls. Prior treatment with one or more immunotherapies or chemotherapy was associated with 55 to 69% lower neopterin concentration, which was statistically significant ($P = .019$, ANOVA), but the range of values for each group was large. None of the four children with the highest neopterin had elevated cerebrospinal fluid total white blood cell counts.

Neopterin was not correlated with neurologic severity score on the Opsoclonus-Myoclonus Evaluation Scale (Table 2). Although each of the four children with the highest neopterin concentrations was in the moderate or severe opsoclonus-myoclonus category, several children with moderate or severe opsoclonus-myoclonus had normal neopterin levels. Neopterin did not correlate with the duration of neurologic symptoms, but none of the children with the highest levels had opsoclonus-myoclonus for more than a few years (Figure 2).

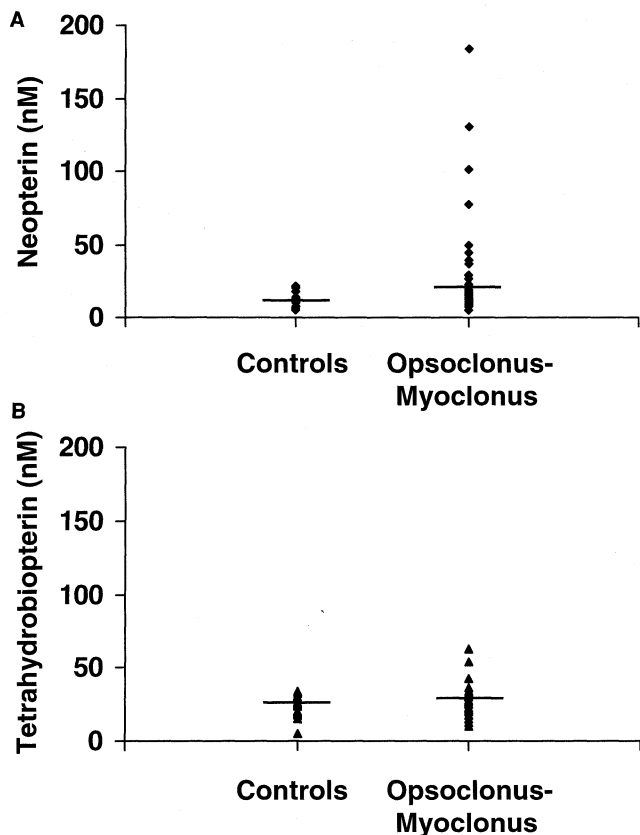


Figure 1. Cerebrospinal fluid concentration of neopterin (A) and tetrahydrobiopterin (B) in controls and opsoclonus-myoclonus. The horizontal line indicates the mean, which was significantly higher in opsoclonus-myoclonus for neopterin ($P = .008$, t -test) but not tetrahydrobiopterin.

Because both neopterin and tetrahydrobiopterin are derived from dihydroneopterin triphosphate, we computed the ratio of the two and also checked for a statistical correlation (see Table 2). There was no significant difference between groups. Twelve children with opsoclonus-myoclonus but only one control had a ratio > 1 , with a top value of 6.55. There was a significant correlation between neopterin and tetrahydrobiopterin in opsoclonus-myoclonus but not in controls, suggesting that tetrahydrobiopterin also increased just enough to affect this correlation.

Table 2. Correlations of Neopterin in Opsoclonus-Myoclonus

Parameter	r	P
Tetrahydrobiopterin	.55	.0002*
Prior therapy	-.46	.026*
Gender	.31	.049
Age	-.28	.083
Total score	.28	.086
Opsoclonus-myoclonus duration	-.20	.22
Opsoclonus-myoclonus etiology	.18	.42

*Statistically significant, $p < .05$.

In controls, there were no significant correlations between neopterin and age or gender.

Data are Pearson correlations.

Neopterin Subgroups in Opsoclonus-Myoclonus

The range of the data in opsoclonus-myoclonus suggested two distinct populations: one that is concordant in neopterin levels with the controls and one whose neopterin levels lie completely out of the control range. To determine if their clinical characteristics differ, we divided the opsoclonus-myoclonus group at 2 SD above the control mean into a "high neopterin" subgroup and a "normal neopterin" subgroup (Figure 3). Thirteen of 40 children with opsoclonus-myoclonus, but none of the controls, had a neopterin concentration > 2 SD above the control mean ($P = .005$, Fisher's exact test).

In the high neopterin subgroup, the neopterin-to-tetrahydrobiopterin ratio was fourfold higher than in the normal neopterin subgroup. Neurologic severity (total score) was 67% higher. In contrast, both patient age (-44%) and the duration of opsoclonus-myoclonus (-65%) were significantly lower. These data indicate that children with higher neopterin values tended to be more severe and more acute in the course of their illness. The high neopterin subgroup also had significantly more females ($P = .02$, Fisher exact test).

Longitudinal Study

In children who underwent repeat lumbar puncture ($n = 17$), immunotherapy (Figure 4) was associated with a 49% reduction in neopterin ($P = .021$, paired t -test). Total score dropped by 53%, which was significant ($P = .016$). There was no significant change in the tetrahydrobiopterin concentrations or the neopterin-to-tetrahydrobiopterin ratio.

Ten of the 15 patients who were videotaped after treatment showed clinical improvement in total score; only those

Table 1. Effect of Clinical Variables on Neopterin in Opsoclonus-Myoclonus

Variable	Subgroup	n	Mean \pm SEM (Range)	P
Etiology	No tumor	24	37.79 \pm 8.98 (5-183)	.29
	Tumor	16	22.13 \pm 4.39 (10-77)	
Gender [†]	Males	17	15.59 \pm 2.42 (5-49)	.027*
	Females	23	38.09 \pm 9.29 (7-183)	
Prior treatment	None	6	58.33 \pm 21.19 (10-131)	.019 [‡]
	Monotherapy	14	18.57 \pm 3.62 (7-49)	
	Combination therapy	20	26.55 \pm 8.52 (5-183)	

Data are from a cross-sectional study of initial lumbar puncture.

*Statistically significant by t -test. When the female patient with the highest neopterin level was dropped, $P = .038$.

[†]In controls, there was no significant neopterin difference between males (11.13 ± 1.65 , $n = 8$) and females (13.27 ± 1.72 , $n = 11$), $P = .39$, t -test.

[‡]Analysis of variance linear trends ($F_{1,37} = 5.98$). On Duncan's Multiple Range Test, the group without prior treatment was significantly different than either treatment group ($P < .05$).

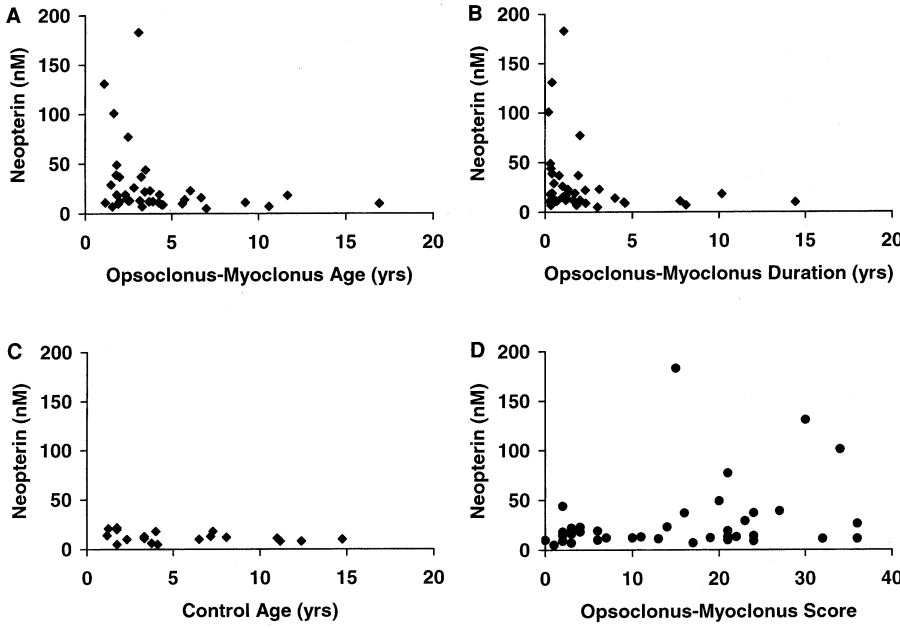


Figure 2. Relation of patient age (A and C), opsoclonus-myoclonus duration (B), and neurologic severity score (D) to neopterin concentration. There were no linear relations, but neopterin was elevated in the more acute and severe cases.

with already low initial scores did not. Eight had reduced neopterin. The largest changes in neopterin concentration were in children with the greatest clinical improvement.

ACTH-Treated Subgroup

Our small sample size for individual types of immunotherapy lacks statistical power for secondary analysis except for ACTH. In seven children treated with ACTH (Figure 5) in combination with other agents, the mean neopterin con-

centration fell by 64% ($P = .034$). The total score dropped by 61%, which was highly significant ($P = .0063$).

Case Studies

Children with the highest neopterin values who went on to treatment warrant further comment. In a 31-month-old child whose opsoclonus-myoclonus began at age 2 years, the initial neopterin was elevated at 101 nM and tetrahydrobiopterin was normal at 54 nM. She had not received any

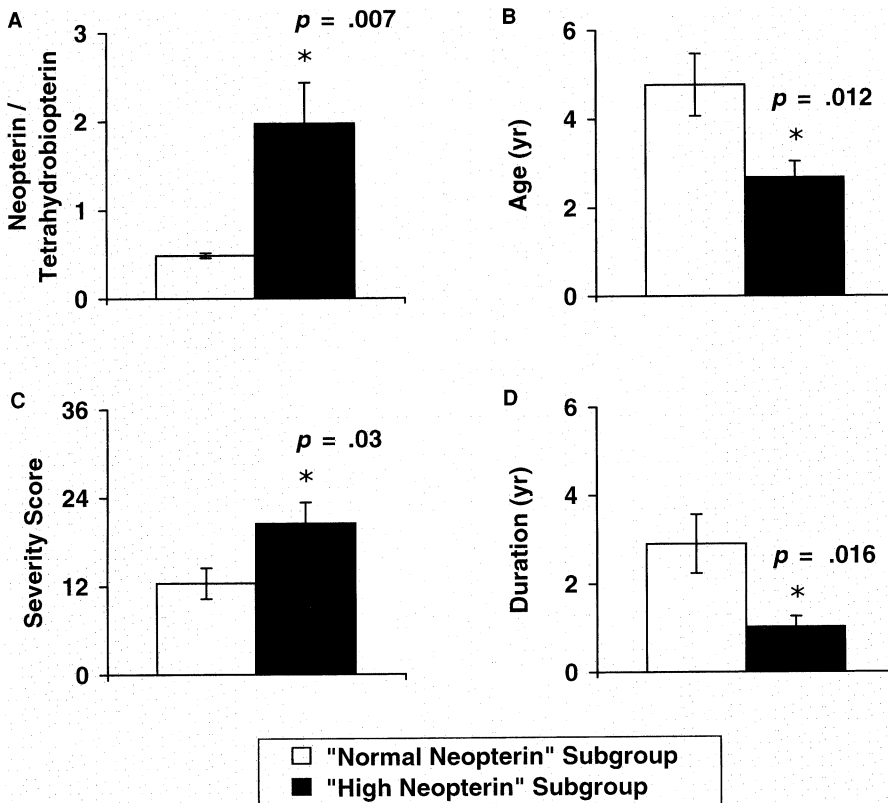


Figure 3. Differences in two subgroups of opsoclonus-myoclonus as defined by > 2 SD above the control mean for cerebrospinal fluid neopterin. In the high neopterin subgroup ($n = 13$), the neopterin-to-tetrahydrobiopterin ratio (A) and neurologic severity score (C) were significantly higher, whereas the patient age (B) and duration of opsoclonus-myoclonus (D) were significantly lower than in the normal neopterin subgroup ($n = 27$) by *t*-tests.

□ "Normal Neopterin" Subgroup
 ■ "High Neopterin" Subgroup

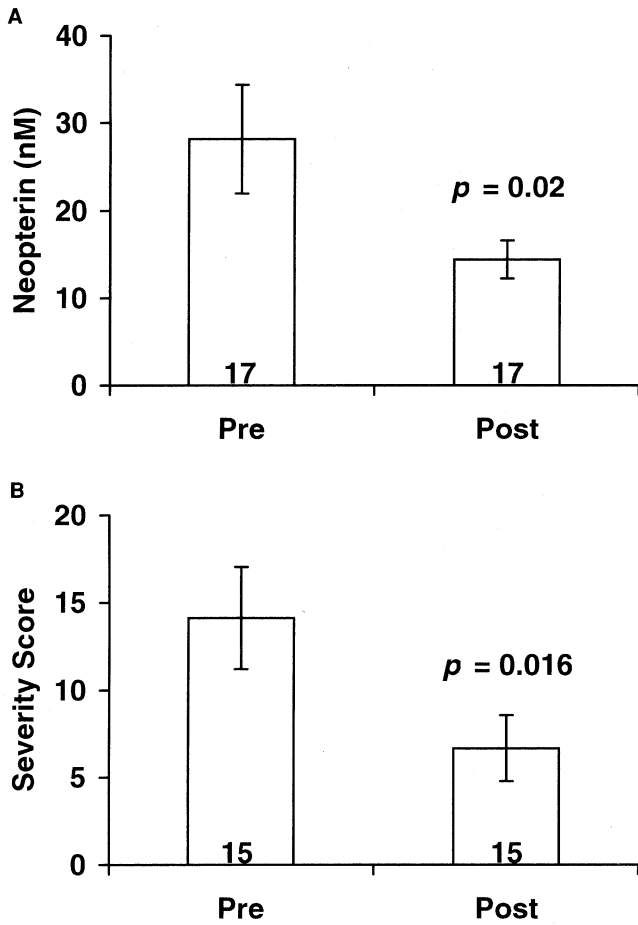


Figure 4. Effect of various immunotherapies collectively on cerebrospinal fluid neopterin (A) and neurologic severity score (B). The *n* values are given at the base of the bars. Treatment significantly lowered neopterin and severity by paired *t*-tests.

prior immunotherapy, and no tumor had been found. Then, on the combination of ACTH, intravenous immunoglobulin, and cyclophosphamide, a complete neurologic remission was

achieved, with a reduction in her total score from 34 to 2. A second lumbar puncture was performed 8 weeks after completion of cyclophosphamide, 6 weeks after ACTH was discontinued, and 4 weeks after the last intravenous immunoglobulin infusion. Neopterin was 33 nM and tetrahydrobiopterin was 38 nM; both were normal.

A 30-month-old girl, with a history of neuroblastoma resection, was evaluated 2 years after opsoclonus-myoclonus presentation while on intravenous immunoglobulin and cyclophosphamide. Her total score was 21, and her neopterin was elevated at 77 nM. After high-dose ACTH was added to her treatment regimen, she improved dramatically, with a drop in total score to 3, and the neopterin fell to 19 nM. Tetrahydrobiopterin remained normal and unchanged at 33 nM.

A 22-month-old boy, whose opsoclonus-myoclonus began at 18 months of age, was evaluated after two treatments with intravenous immunoglobulin. No tumor had been found. On the initial lumbar puncture, when his total score was 20, the neopterin level was elevated at 49 nM and the tetrahydrobiopterin level was normal at 34 nM. High-dose ACTH and cyclophosphamide were added to his therapeutic regimen. A year later, while still on ACTH and 6 months after completion of cyclophosphamide, the neopterin fell to 11 nM, and the tetrahydrobiopterin remained about the same at 36 nM.

DISCUSSION

The main finding in pediatric opsoclonus-myoclonus was increased cerebrospinal fluid concentrations of neopterin, an indicator of activation within the cell-mediated immune system.¹⁸ Neopterin was more elevated in acute and untreated patients, and the magnitude of increase was comparable to that seen in central nervous system viral infections.¹¹ Our control data agree with published normative data.^{10,19}

Cerebrospinal fluid neopterin elevations may be due to intrathecal immune response.²⁰ Neopterin rises after allo-

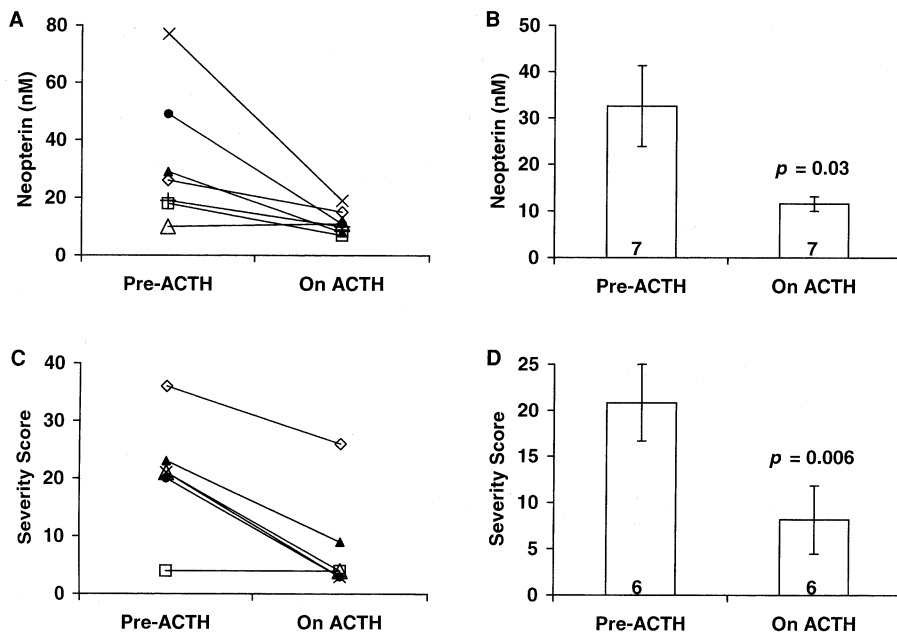


Figure 5. Effect of adrenocorticotropic hormone (ACTH) combination therapy on cerebrospinal fluid neopterin concentration (A and B) and neurologic severity score (C and D). The patient with the hollow square in the upper figure was not scored and is absent from the lower figure. Both for neopterin and severity, the means (\pm standard error of the mean) shown to the right were significantly lower at the time of the second lumbar puncture by paired *t*-test. The *n* values are given at the base of the bars.

genic T-cell activation but need not reflect T-subset imbalance (inversion of the CD4-to-CD8 ratio) or an increase in human leukocyte antigen DR⁺.¹¹ Its production in monocytes and macrophages is inducible by cytokines, especially interferon- γ released by activated T cells. In vitro, T cells, but not B cells or natural killer cells, are induced to form neopterin by interferon- γ ,⁸ which is high in the cerebrospinal fluid of the same patients in whom neopterin is elevated.²⁰ Neopterin enhances reactions involving oxygen and chlorine free radicals, which participate in the effector functions of monocytes and macrophages. Its cerebrospinal fluid concentration is increased in various central nervous system infections and autoimmune diseases.^{8,10,21}

These data support our recent cerebrospinal fluid lymphocyte immunophenotyping studies, which demonstrated both T-cell and B-cell abnormalities in opsoclonus-myoclonus using flow cytometry.²² We found an elevated percentage of activated T cells and $\gamma\delta$ T cells, as well as T-subset imbalance. The abnormalities correlated with neurologic severity. Immunophenotyping studies confirm that the cell types purported to be involved in the neopterin effect are present in opsoclonus-myoclonus cerebrospinal fluid.

As to the normal tetrahydrobiopterin in opsoclonus-myoclonus, it should be noted that tetrahydrobiopterin is a necessary cofactor for aromatic amino acid monooxygenases in the metabolism of brain monoamines, such as serotonin and dopamine. Most biopterin in the brain exists as tetrahydrobiopterin and is concentrated in monoaminergic neurons. Our study indicates that the low cerebrospinal fluid 5-hydroxyindoleacetic acid and homovanillic acid we found previously in a subgroup of opsoclonus-myoclonus²³ probably are not due to a reduction in tetrahydrobiopterin.

These data support a role for cellular immune activation, specifically T-cell activation, in acute opsoclonus-myoclonus. Our study also warns against discounting the possibility of a significant central nervous system inflammatory process in children with opsoclonus-myoclonus who have normal cerebrospinal fluid cell counts. Neopterin was not a sensitive biomarker of disease activity in opsoclonus-myoclonus because some children with opsoclonus-myoclonus, despite neurologic abnormalities, did not have an elevated neopterin concentration, perhaps as a consequence of prior immunotherapy. It also cannot be used to differentiate tumor and nontumor etiologies of opsoclonus-myoclonus. If cerebrospinal fluid neopterin has clinical utility in pediatric opsoclonus-myoclonus, it is at the initial diagnostic evaluation of children with acute, severe presentation.

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