Cerebrospinal Fluid Protein Concentration in Pediatric Patients

Defining Clinically Relevant Reference Values

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Objectives: To define clinically relevant reference ("normal") values for cerebrospinal fluid (CSF) protein concentrations in pediatric patients who were evaluated for meningitis by traditional criteria and by enterovirus–polymerase chain reaction (EV-PCR).

Design and Patients: A cohort of 906 consecutive pediatric patients to receive CSF analysis at St Louis Children’s Hospital, St Louis, Mo, from June 1, 1998, to December 31, 1998, was studied for clinical and laboratory data. Age-dependent CSF protein concentrations were then derived from a reference group of 225 patients in whom meningitis and other neurologic diseases were excluded by traditional clinical or laboratory criteria (excluding EV-PCR). Available CSF samples from 132 patients of the reference group were subsequently tested for EV-PCR.

Results: In the reference group, the CSF protein concentration was highest and most variable in neonates, with a maximum of approximately 1.0 g/L. Cerebrospinal fluid protein concentration decreased rapidly to a nadir by 6 months and remained low throughout childhood, rarely exceeding 0.3 g/L and, finally, increasing in adolescence toward adult values. Enterovirus–polymerase chain reaction was positive in CSF of 11% of the reference group, with EV-PCR–positive patients having significantly higher CSF protein concentrations than EV-PCR–negative patients aged between 4 months and 14 years.

Conclusions: Reference values for CSF protein exhibit a characteristic age dependence in pediatric patients. Continued standard use of adult reference values in the pediatric population is inappropriate. The unexpected finding of a positive EV-PCR in patients not diagnosed with meningitis by traditional criteria further emphasizes the importance of selecting the most clinically relevant reference group for age and other variables when defining normal laboratory values.


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Identification of a pathologic process by lumbar puncture and cerebrospinal fluid (CSF) analysis depends on having well-established reference ("normal") values. In pediatrics, defining a normal reference range for CSF parameters is complicated by several factors. Although many clinical laboratories use adult reference values for pediatric patients, previous studies of children demonstrate that CSF parameters, such as leukocyte count and protein level, normally vary with age. Significant differences between these studies exist in the absolute values, timing, and rate of these age-dependent changes. These discrepancies most likely relate to different biases inherent in the selection criteria for "normal" subjects included in the studies. Defining accurate reference ranges for CSF parameters in children requires selection of an appropriate population sample. For ethical reasons, studies of CSF rarely use completely healthy children, resulting in normal values obtained from patient populations that are not truly normal. In fact, from a clinical standpoint, healthy subjects may not represent the most desirable reference group, as clinical decisions often rely on distinguishing between a serious pathologic process and a benign self-limited condition, both of which may theoretically alter CSF parameters. Thus, the most clinically relevant reference group may vary depending on the specific clinical scenario.

The most common indication for lumbar puncture is to evaluate for evidence of infection of the nervous system. Meningitis is traditionally diagnosed based on a combination of clinical and laboratory findings, with CSF leukocyte count, Gram stain, and cultures usually representing the most critical laboratory data. Recently, polymerase chain reaction (PCR) technology has introduced a more sensi-
PATIENTS AND METHODS

Clinical and laboratory data were obtained from the medical records of all 906 patients to receive CSF analysis at St Louis Children’s Hospital, St Louis, Mo, from June 1, 1998, to December 31, 1998. The study was designed prospectively prior to this period to ensure an inclusive population sample with minimal selection bias and allow optimal processing of extra CSF samples for PCR analysis by the clinical laboratory. The medical records of all patients receiving CSF analysis were identified and reviewed retrospectively using standardized criteria for classifying patient diagnoses. Figure 1 outlines the major exclusionary and diagnostic categories used to derive the reference group. Patients with a history of prematurity or patients who had a traumatic lumbar puncture were automatically excluded from the reference group. A history of prematurity was defined as fewer than 37 weeks’ gestational age in patients who were younger than 12 months at the time of lumbar puncture. A traumatic lumbar puncture was defined as either (1) the report of blood in the CSF sample by the physician in the procedure note or by the laboratory, or (2) more than 200 × 10^6 erythrocytes per liter in the leukocyte count.

The targeted reference (normal) group for this study comprised otherwise healthy children who were initially seen with an indication for lumbar puncture, but were ultimately found to have no evidence of acute or chronic neurologic or systemic disease. Patients were excluded from the reference group if they were found to have clinical or laboratory evidence of any active or past neurologic disorder, eg, stroke, hydrocephalus, intracranial infection, seizures or epilepsy, static encephalopathy, head trauma, demyelinating disease, or disorders of the peripheral nerve or muscle. Patients with acute or chronic systemic disease, eg, cancer, bacteremia or sepsis, and hematologic or rheumatologic disorders, were also excluded. Based on traditional criteria for meningitis, positive evidence of CNS infection consisted of an appropriate clinical presentation in combination with CSF pleocytosis or cultures positive for virus or bacteria in the CSF. Cerebrospinal fluid pleocytosis was defined from previously reported age-dependent reference values for CSF leukocyte counts: greater than 22 × 10^6 leukocytes per liter for neonates aged between 0 and 4 weeks, greater than 15 × 10^6 leukocytes per liter for infants aged between 4 and 8 weeks, and greater than 7 × 10^6 leukocytes per liter for all patients older than 8 weeks.6,7

Two hundred twenty-five patients were assigned to the reference group by the aforementioned criteria without knowledge of the EV-PCR. Cerebrospinal protein concentration was measured using a protein analyzer (Vitros model 250; Johnson & Johnson Clinical Diagnostics Inc, Rochester, NY). One hundred thirty-two CSF samples from the reference group were tested for EV by reverse transcription PCR using a commercial colorimetric microwell detection assay (Dia Sorin Inc, Stillwater, Minn). The CSF of 93 patients was not tested for EV-PCR for 1 of 2 reasons. In some cases, there was an insufficient sample. In the remaining cases, since a disproportionate percentage of patients were younger than 4 months, a subset of samples from this age group was randomly selected for EV-PCR testing. For initial analysis of the age dependence of CSF protein concentration, patients were first categorized according to the following age groups: 0 to younger than 2 weeks, 2 weeks to younger than 1 month, monthly for the remainder of the first year (ie, 1-2 months, >2-3 months, >3-4 months, . . .), and annually (ie, >1-2 years, >2-3 years, >3-4 years, . . .) thereafter.

Based on visual inspection and preliminary statistical analysis, age groups were subsequently condensed into 4 categories (0 to <2 months, 2 to <4 months, 4 months to <14 years, and 14-18 years), which were analyzed for statistical differences using nonparametric analysis of variance (Kruskal-Wallis test) and nonparametric multiple comparisons (Dunn test). The effect of the EV-PCR on CSF protein concentration in these 4 age categories was tested using the Mann-Whitney test. χ^2 Test analysis was used to test for seasonal variation in EV-PCR positivity. This study was conducted in accord with a protocol approved by the Human Studies Committee of Washington University, St Louis, Mo. Informed consent from patients was waived based on the following grounds. The study did not alter patient management in any way, eg, in the decision to perform the lumbar puncture, the amount of CSF sample collected, or the particular tests ordered by the physician. Specifically, the physician ordering these tests decided to obtain testing for CSF protein concentration for clinical indications only, independent of this study. Cerebrospinal fluid subsequently used in this study for EV-PCR analysis was tested anonymously, using only samples that remained after the physician-ordered tests were completed and that were to be discarded.

RESULTS

AGE DEPENDENCE OF CSF PROTEIN CONCENTRATION

Independent of the EV-PCR, 225 patients met the criteria for the reference group (Figure 1). The indications for lumbar puncture in these patients were: (1) to rule out meningitis (n=223), (2) to rule out multiple sclerosis (n=1), and (3) to evaluate chronic headaches (n=1). The final diagnoses of the reference group consisted of otitis media or upper respiratory tract infection or other
respiratory illness (n=62), gastroenteritis or other gastrointestinal tract illness (n=21), skin or other localized infection (n=17), nonspecific febrile illness or fever without a determined source (n=71), benign headache syndrome (n=33), conversion disorder (n=3), normal patient or no clinical diagnosis (n=14), and other (n=4).

Figure 2, top left and bottom left, shows the values of CSF protein concentration for all patients in the reference group as a function of age. As summarized in Table 1, patients between ages 0 and 2 months had a higher mean concentration, accompanied by greater variability of protein values, compared with older patients. The patients’ mean CSF protein concentration dropped rapidly between the second and fourth month of life and reached a minimum of less than 0.20 g/L by approximately 6 months. Protein values remained low throughout childhood and then increased during adolescence toward the adult range, although there were only a limited number of patients older than 14 years. For patients aged between 4 months and 14 years, the CSF protein concentration was rarely greater than 0.30 g/L (3 [4%] of 77 patients in this age group, with 2 of these 3 patients having a positive EV-PCR—see the “Effect of EV-PCR Testing on Patient Selection and CSF Protein Concentration” section below) and never higher than 0.35 g/L. After dividing the data into the 4 age groups of 0 to younger than 2 months, 2 to younger than 4 months, 4 months to younger than 14 years, and 14 to 18 years (Table 1), multiple comparisons statistical analysis found the first 3 groups to be statistically significantly (P < .001) different from each other. The adolescent group (14-18 years) was statistically significantly (P < .001) different from the first age group (0 to <2 months), but was not statistically different from the other 2 groups (2 to <4 months and 4 months to <14 years) by nonparametric testing despite having nonoverlapping 95% confidence intervals of the mean.

**EFFECT OF EV-PCR TESTING ON PATIENT SELECTION AND CSF PROTEIN CONCENTRATION**

One hundred thirty-two patients from the reference population were tested for EV-PCR in the CSF. Fourteen (10.6%) of the 132 had a positive EV-PCR in the CSF, despite not meeting the traditional criteria for meningitis. Although these EV-PCR–positive patients had CSF leukocyte counts within the normal range, 6 of 14 had
COMMENT

Table 1. Cerebrospinal Fluid (CSF) Protein Values of 225 Reference Patients by Age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>n</th>
<th>CSF Protein Concentration, g/L</th>
<th>95% CI* of Mean</th>
<th>Median</th>
<th>Range</th>
<th>90th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to &lt;2 mo†‡</td>
<td>99</td>
<td>0.59±0.21</td>
<td>0.55-0.63</td>
<td>0.60</td>
<td>0.20-1.10</td>
<td>0.87</td>
</tr>
<tr>
<td>2 to &lt;4 mo†‡</td>
<td>36</td>
<td>0.37±0.15</td>
<td>0.32-0.42</td>
<td>0.37</td>
<td>0.09-0.78</td>
<td>0.55</td>
</tr>
<tr>
<td>4 mo to &lt;14 y‡</td>
<td>77</td>
<td>0.17±0.06</td>
<td>0.16-0.18</td>
<td>0.15</td>
<td>0.09-0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>14-18 y</td>
<td>13</td>
<td>0.26±0.06</td>
<td>0.23-0.29</td>
<td>0.28</td>
<td>0.17-0.36</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*CI indicates confidence interval.
†P<.001 compared with all other age groups by nonparametric multiple comparisons testing.
‡P<.001 compared with all other age groups, except the 14- to 18-year-old group.

Table 2. Effect of Enterovirus–Polymerase Chain Reaction (EV-PCR) Test Results on Cerebrospinal Fluid (CSF) Protein Concentration

<table>
<thead>
<tr>
<th>Age Group</th>
<th>EV-PCR–Positive Patients (n = 14)</th>
<th>EV-PCR–Negative Patients (n = 118)</th>
<th>Total (N = 132)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to &lt;2 mo†‡</td>
<td>Mean ± SD CSF Protein Concentration, g/L</td>
<td>Median ± SD CSF Protein Concentration, g/L</td>
<td>Median ± SD CSF Protein Concentration, g/L</td>
</tr>
<tr>
<td>2 to &lt;4 mo†‡</td>
<td>0.48±0.11</td>
<td>0.37-0.59</td>
<td>0.48</td>
</tr>
<tr>
<td>4 mo to &lt;14 y‡</td>
<td>0.36±0.07</td>
<td>0.28-0.44</td>
<td>0.38</td>
</tr>
<tr>
<td>14-18 y</td>
<td>0.24±0.06</td>
<td>0.20-0.28</td>
<td>0.23</td>
</tr>
</tbody>
</table>

An absolute neutrophil count of more than 1 × 10⁹/L, with a mean (±SD) of 2.6 ± 1.6 × 10⁹/L. The incidence of positive EV-PCR in the reference group was significantly higher (P<.01, χ² test analysis) during summer months (12 of 70 from June through mid-September) compared with the fall–early winter months (2 of 62 from mid-September through December).

Table 2 gives the age distribution and CSF protein values relative to the EV-PCR. In the age group of 4 months to younger than 14 years, patients with positive EV-PCR in the CSF had a significantly elevated CSF protein level compared with EV-PCR–negative patients of similar age (P<.01, Mann-Whitney test). Since EV-PCR–positive patients represented a minority of the total group, CSF protein parameters for the overall reference group were not significantly different compared with EV-PCR–negative patients alone (Table 2 and Figure 2).

Several previous studies have attempted to define normal values for various CSF parameters.1-5,8,10 As healthy children are rarely used for such studies, a recognized problem involves selection of an appropriate reference group. Differences in CSF reference data exist between studies and most likely relate to the study population and exclusion and inclusion criteria used. The most appropriate reference group may vary depending on the particular clinical scenario and the specific clinical questions in mind. The most common indication for lumbar puncture is to rule out meningitis. Pediatric patients evaluated for meningitis often have nonspecific symptoms, such as isolated fever in a neonate or young infant. In this clinical scenario, an appropriate reference group would consist of patients who had nonspecific symptoms consistent with meningitis but, ultimately, had meningitis ruled out.

AGE-SPECIFIC CSF PROTEIN VALUES IN THE PEDIATRIC POPULATION

Our study examined CSF protein values in a pediatric reference group in which meningitis had been excluded based on traditional criteria. The use of a large unselected cohort of patients receiving lumbar puncture minimized the problems of selection bias in generating the reference group. Most previous studies agree on a general trend of age-dependent changes in CSF protein concentration, but differ significantly in the absolute values, timing, and rate of these changes.1,5,12,13 Our study confirms this characteristic age dependence of CSF protein. In the neonatal period, CSF protein values were extremely variable with an upper limit of approximately 1.0 g/L. By comparison, a prior study reported an even higher range of up to 1.7 g/L for full-term neonates.7 As even a small amount of blood contamination may significantly alter CSF values,11 a likely source for this discrepancy is the more stringent exclusionary criteria for traumatic taps in our study. Over the first few months of life, CSF pro-
tein values rapidly declined, an effect often attributed to decreasing permeability of the blood-brain barrier, and reached a minimum by approximately 6 months of age in our study. This rate of decline in infancy agrees with classic studies by Widell, but contrasts slightly with other studies that found a slower rate of decline, reaching a minimum when the subject is approximately 2 years of age. Throughout the remainder of childhood up to age 14 years, CSF protein concentration remained extremely low, rarely being higher than 0.30 g/L and never higher than 0.35 g/L in our study. During adolescence, CSF protein concentration gradually increased toward adult levels by uncertain mechanisms. Thus, based on our data and previous studies, CSF protein levels above 1.0 g/L in neonates and 0.35 g/L in preadolescent children should raise concerns about a pathologic process.

Overall, these findings support the use of age-specific reference ranges for CSF protein in pediatric patients, such as those outlined in Table 1. Although some data supporting this contention have existed for over 40 years, one based on our clinical experience and perception, few clinicians are sufficiently aware of these age-dependent differences, especially the lower CSF protein values of preadolescent children. Similarly, many clinical laboratories and popular reference sources cite inappropriately high CSF protein values for children, usually adopting adult maximal values of 0.40 or 0.45 g/L. Based on our study, the continued standard use of adult reference values in the pediatric population seems inappropriate.

IMPLICATIONS OF EV-PCR TESTING FOR REFERENCE GROUP COMPOSITION AND CSF PROTEIN VALUES

Although the patients in our reference group were not diagnosed with meningitis based on traditional criteria of clinical course, CSF leukocyte count, and CSF culture, a notable percentage of the reference group had a positive EV-PCR. While these results could be false positive, this is unlikely for several reasons. Enterovirus–polymerase chain reaction assays have been estimated to have a specificity of at least 90% compared with viral culture, and the true specificity is probably much higher. The incidence of a positive EV-PCR was higher during the summer months, the peak of enteroviral season. Patients with positive EV-PCR had moderately elevated CSF protein levels and some had elevated absolute neutrophil counts, indicating that they did, indeed, have EV meningitis that went undiagnosed. Finally, EV meningitis has previously been reported to occur without CSF pleocytosis.

The unexpected detection of meningitis in our original reference group has important implications concerning the interpretation of normal laboratory values in general, and supports the concept of defining the most clinically relevant reference group. Patients older than 4 months with positive EV-PCR had significantly higher CSF protein levels compared with EV-PCR–negative patients. Since EV-PCR–positive patients constituted a minority of the reference group, inclusion of these patients with higher protein values only had a minor effect on the overall CSF protein reference values in this study. However, previous studies of CSF protein reference values lacking PCR data likely also included patients with meningitis without pleocytosis, the effect of which is unknown. Ultimately, the practical effect of including such patients in the reference group depends again on the particular clinical scenario. From a biological perspective, the reference group that excludes these patients approaches physiological normality more closely. From a clinical standpoint, use of a potentially higher protein reference range that includes these patients may be more appropriate for issues of clinical management or outcome. Since the EV-PCR–positive patients in this study had benign, self-limited clinical courses, the retrospective diagnosis of meningitis in these patients was probably of little practical consequence. Independent of which reference group is used, this study emphasizes the importance of critically analyzing how laboratory reference values are defined and applied in clinical practice.

Accepted for publication March 20, 2000.

We thank Phillip Dodge, MD, for providing the impetus for this study and Edwin Trevathan, MD, MPH, for critically analyzing the manuscript from an epidemiological standpoint.

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