Cerebrospinal Fluid Protein Concentrations in Children: Age-related Values in Patients without Disorders of the Central Nervous System

Daniel Biou,* Jean-François Benoist, Claire Nguyen-Thi Xuan Huong, Philippe Morel, and Martine Marchand

Background: The published reference values for cerebrospinal fluid (CSF) total protein concentrations in children suffer from two major drawbacks: (a) the age-related range often is too broad when applied to the steeply falling concentrations in early infancy; and (b) no values have been published for widely used dry chemistry methods.

Methods: We conducted a 2-year retrospective survey of CSF results obtained in a children’s hospital with a dry chemistry-based method set up on the Vitros 700 analyzer.

Results: The data related to ambulatory children up to 16 years of age and term neonates with no clinical or biological signs of brain disease (n = 1074). Seven age groups with significantly different CSF protein values were identified, and their age-related percentiles (5th, 50th, and 95th) were determined. On the basis of the upper 95th percentile, from age 0 to 6 months the CSF protein concentrations fell rapidly from 1.08 to 0.40 g/L. A plateau (0.32 g/L) was reached from age 6 months to 10 years, followed by a slight increase (0.41 g/L) in the 10–16 years age range.

Conclusions: These results imply that CSF total protein concentrations in the pediatric setting, particularly in infants, must always be interpreted with regard to narrow age-related reference values to avoid false-positive results.

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Measurement of total protein in cerebrospinal fluid (CSF) is used mainly to detect various central nervous system (CNS) diseases associated with either increased permeability of the blood-CSF barrier or increased intrathecal immunoglobulin synthesis. The largest increases are seen in bacterial meningitis, with smaller increases in other inflammatory, traumatic, or tumoral disorders and after intracerebral hemorrhage (1).

In the pediatric setting, CSF testing must be done rapidly and with a small sample volume. The CSF protein assay on the Vitros 700 (formerly the Kodak Ektachem; now available from Ortho-Clinical Diagnostics) meets these conditions. However, the central 95% percentile reference interval given by the manufacturer (120–600 mg/L) and determined by Lott and Warren (2) apparently is based on a small number of samples from adults only. The authors claimed there were no statistically significant age-related differences. However, because of the immaturity of the blood-CSF barrier in fetuses and neonates, it is well known that CSF protein concentrations in newborn infants, and especially premature newborns, are higher than in children and adults (3).

The published reference values for CSF total protein concentrations suffer from two major drawbacks: (a) the values are method dependent (type of dye, precipitating agent, and calibrator); and (b) the age-related range often is too broad when applied to the steeply falling CSF protein concentrations in newborn infants (Table 1) (3–5).

Curiously, since the first report by Lott and Warren (2), no other age-related reference intervals have been published for CSF protein concentrations determined by dry chemistry methods in children. We therefore conducted a 2-year retrospective survey of CSF results obtained in a children’s hospital.

Patients and Methods

Patients used for reference data. For obvious ethical reasons, CSF was not sampled in healthy children. We therefore used data on CSF specimens obtained between May 1997 and May 1999 by lumbar puncture from term infants (gestational age, 38–41 weeks) and children (up to 16
years) consulting Robert Debré Children’s Hospital for suspected CNS disease (n = 2182). Also included in this study were CSF samples from term newborns hospitalized in the maternity or neonatology departments (n = 512). All specimens from children hospitalized in other wards were discarded to exclude the many CNS disorders and iatrogenic complications that can increase CSF protein concentrations (1).

All specimens with the following features were excluded: pigmented, turbid, purulent, or bloody aspect; presence of an erythrocyte pellet after centrifugation; abnormal erythrocyte or leukocyte count; positive Gram staining; positive microbiological culture; positive bacterial antigens; antibiotic therapy before CSF sampling; and samples from patients hospitalized for >1 day. The remaining 1074 specimens (1013 from ambulatory children and 61 from hospitalized newborns) were used to define the reference population.

**Patients used for method comparison.** All unselected consecutive CSF specimens obtained during a 2-month period (n = 337) from hospitalized or consulting patients were included in the study to obtain CSF protein values covering a very wide range (0.1–2 g/L, the upper limit of linearity). CSF samples large enough for duplicate assays were centrifuged and assayed immediately with two analyzers (see below).

**ASSAYS METHODS**

**Vitros 700.** The Vitros 700 CSF protein determination is based on biuret colorimetry, but with decreasing reflectance density (670 nm) of the Cu²⁺-azoic dye complex when Cu²⁺ is complexed by the peptide bond (2). The instrument is calibrated with a mixture of a polymer and purified human protein. An endpoint measurement is made 5 min after the addition of 10 μL of the CSF specimen to the slide. The respective between-run imprecision for two controls (Liquid Performance Verifier 1 and 2; Vitros) was as follows: n = 60; x = 0.69 g/L; CV = 5.3%; and n = 59; x = 1.46 g/L; CV = 3.4%.

**Hitachi 911 (Roche Diagnostics).** In this method, CSF protein reacts with a pyrogallol red-molybdate complex to form a blue-purple complex that absorbs at 600 nm (6). In the protocol provided by the manufacturer (Biotrol Diagnostic), an endpoint measurement is made 10 min after the addition of 20 μL of the CSF specimen. The calibrator is bovine albumin. The between-run imprecision for two serum con-

<table>
<thead>
<tr>
<th>Age</th>
<th>Mean ± SD* (range)</th>
<th>Range*</th>
<th>Mean (range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term newborn</td>
<td>0.40–1.20</td>
<td>0.63 (0.32–2.40)</td>
<td>0.90d (0.20–1.70)</td>
</tr>
<tr>
<td>0–1 week</td>
<td>0.77 ± 0.16 (0.45–1.09)</td>
<td>0.47 (0.27–0.65)</td>
<td></td>
</tr>
<tr>
<td>1–2 weeks</td>
<td>0.66 ± 0.16 (0.51–1.01)</td>
<td>0.20–0.80d</td>
<td></td>
</tr>
<tr>
<td>2–3 weeks</td>
<td>0.29 ± 0.04 (0.23–0.37)</td>
<td>0.15–0.45j</td>
<td></td>
</tr>
<tr>
<td>3–6 months</td>
<td>0.27 ± 0.07 (0.17–0.35)</td>
<td>0.15–0.80d</td>
<td></td>
</tr>
<tr>
<td>6–12 months</td>
<td>0.22 ± 0.05 (0.16–0.31)</td>
<td>0.20–0.80d</td>
<td></td>
</tr>
</tbody>
</table>

*From Statz and Feigenhauer (3).
*From Soldin et al. (4).
*From Klein and Marcy (5).
*Age range, newborn to 1 week.
*Age range, 0–2 weeks.
*Age range, 1–4 weeks.
*Age range, 0–4 weeks.
*Age range, 2–4 weeks.
*Age range, 1–3 months.
*Age range, 1 month to adult.
trols (Lyphochek; Bio-Rad) was as follows: n = 63; x = 0.32 g/L; CV = 6.2%; and n = 63; x = 0.70 g/L; CV = 2.9%.

STATISTICAL ANALYSIS

Because we could not predict the distribution of the population in each age group, nonparametric tests were used to estimate the statistical significance of the reference ranges. The Kruskal–Wallis test was used to test for statistical significance of differences in CSF protein values between the all age groups. When significance was found, pairwise comparisons between successive age groups were made with the Kolmogorov–Smirnov test. When there was no statistically significant difference (P > 0.05), the successive age groups were pooled. The StatView program (SAS Institute), Ver. 5.0, was used for statistical analysis.

RESULTS

REFERENCE INTERVALS

After birth, the distribution of CSF total protein concentrations fell rapidly (Fig. 1A). This fall was maximal during the first 100 days of life, and was much less pronounced thereafter. Over an average 6 months to 1 year age range, a plateau was reached. We divided the data into 11 age groups: 1–8 days, 8–30 days, 1–2 months, 2–3 months, 3–4 months, 4–5 months, 5–6 months, 6 months to 1 year, 1–2 years, 2–10 years, and 10–16 years. Applying the nonparametric Kruskal–Wallis test to the age groups defined above, we found a highly significant age effect on the distribution of CSF protein values. The nonparametric Kolmogorov–Smirnov test was then applied to identify an age effect between successive age groups. The difference was not statistically significant for the three age groups between 3 and 6 months and the three between 6 months and 10 years (results not shown). In contrast, a significant difference was found between all of the other successive paired age groups.

On the basis of these findings, the reference population was again distributed into the following seven age groups: 1–8 days, 8–30 days, 1–2 months, 2–3 months, 3–6 months, 6 months to 10 years, and 10–16 years. The relationship between the 5th, 50th, and 95th percentiles of the CSF protein concentration and the mid point of each age group (4, 19, 46, 76, 136, 4718, and 4749 days) gave a statistical estimate of the age dependence of the reference interval (Fig. 1B) and confirmed the rapid fall between birth and 6 months of age. However, from 10 years onward, a trend toward higher values was found. These successive downward and upward trends in CSF protein...
concentrations were highly significant for each of the above age groups when tested with the Kolmogorov–Smirnov test (Table 2). Inside a given age group, a more precise estimate of the upper limit of the reference interval could be obtained by interpolation of the patient’s age on the 95th percentile plot.

**METHOD COMPARISON**

The equation of the regression line of best fit was $y = 0.802x$ (Vitros) + 0.00039 g/L ($n = 337$; $SE$ of the slope, 0.008; $SE$ of the intercept, 0.004; $r = 0.985$; mean Hitachi = 0.360 g/L; mean Vitros = 0.449 g/L). The values determined by the pyrogallol red method ($y$; Hitachi) correlated well with those obtained by the copper-binding method ($x$; Vitros). However, regression analysis showed a significant proportional negative bias, at the 95% confidence interval, of the pyrogallol method ($y$) compared with the copper-binding method.

**Discussion**

The CSF protein concentration depends on the serum protein concentration and on the permeability of the blood-CSF barrier. Because of the higher permeability of the immature barrier of preterm and term infants, their CSF has higher protein content than that of children and adults. Maturation of the barrier gradually increases after birth, reaching its maximum at the third month of life (3). This was shown by the age distribution of the values in the reference population, with a maximal decrease during the first 6 months of life. The rate of the fall during the first trimester demonstrates the poor predictive performance of the CSF protein test when appropriate narrow age groups are not used in this period.

Given the smallest sample size of our age groups ($n = 26$) and the fact that the theoretical minimal sample sizes required for the respective 97.5th and 95th percentile estimates are 40 and 20 values (1), we defined the upper limit of the present reference intervals as the 95th percentile. On the basis of this upper limit, the physiological maturation of the blood-brain barrier during early infancy is illustrated by a corresponding rapid fall (from 1.08 to 0.60 g/L) between birth and 3 months of age. Thereafter, the decline in CSF protein was less pronounced, reaching a minimal value (0.32 g/L) in the 6 months to 10 years age range. From 10–16 years, a slight increase was observed, in keeping with the significant increase reported by Tibbling et al. (7) in aging adults (from 0.37 ± 0.06 g/L to 0.53 ± 0.13 g/L between 17–30 years and 61–77 years, respectively). In agreement with the above finding, the 95% central reference interval (2.5–97.5) of our oldest age group (10–16 years) was lower (0.10–0.43 g/L) than that of adults (0.12–0.60 g/L), as determined with the same analyzer used by Lott and Warren (2), thus confirming the slight but continuous increase in CSF protein concentrations starting in early adolescence. Nevertheless, given the continuous increase in aging adults, the adult reference interval reported by Lott and Warren (2) seems to be an approximation of the true situation.

In the literature, the reference values for CSF protein often are expressed as gaussian statistics (mean ± SD) and are often based on small samples with no details of value distribution. This approach is at the very least questionable. More worrying is that the age range is often too large [0.15–0.45 g/L above 1 month of age (4)] and thus fails to reflect the rapid fall in physiological CSF protein concentrations during the first 3 months of life. To our knowledge, the most complete report covering the entire period of development through infancy and childhood is from Statz and Felgenhauer (3). Our sample is even larger, enabling us to define narrower reference intervals, particularly for the 1–2 months and 2–3 months age ranges, during which the most rapid decline in CSF protein concentrations occurs. Our data may lead to better diagnostic performance (specificity, sensitivity, positive and negative predictive values, and efficiency) of neonatal CSF protein assays.

When necessary, CSF protein values should be checked with a different chemical method to detect possible interference by drugs used to treat CNS disorders (2). We found a good correlation between the control and routine methods (Hitachi vs Vitros) with, however, significantly lower values in the former. These findings are in agreement with those of Lott and Warren (2), who reported higher results (+26%) with the copper-binding methods (biuret and Vitros) than with a turbidimetric method. In addition, these authors found a good correlation between

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**Table 2. Age-related percentiles of CSF total protein (g/L) in infants and children, as determined by the Vitros method.**

<table>
<thead>
<tr>
<th>CSF total protein, g/L</th>
<th>1–8 days</th>
<th>8–30 days</th>
<th>1–2 months</th>
<th>2–3 months</th>
<th>3–6 months</th>
<th>6 months–10 years</th>
<th>10–16 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>26</td>
<td>76</td>
<td>155</td>
<td>115</td>
<td>66</td>
<td>599</td>
<td>37</td>
</tr>
<tr>
<td>5th Percentile</td>
<td>0.33</td>
<td>0.31</td>
<td>0.27</td>
<td>0.18</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>50th Percentile</td>
<td>0.71</td>
<td>0.59</td>
<td>0.47</td>
<td>0.35</td>
<td>0.23</td>
<td>0.18</td>
<td>0.22</td>
</tr>
<tr>
<td>95th Percentile</td>
<td>1.08</td>
<td>0.90</td>
<td>0.77</td>
<td>0.60</td>
<td>0.40</td>
<td>0.32</td>
<td>0.41</td>
</tr>
<tr>
<td>Range</td>
<td>0.26–1.35</td>
<td>0.26–1.15</td>
<td>0.18–0.86</td>
<td>0.10–0.74</td>
<td>0.10–0.54</td>
<td>0.10–0.44</td>
<td>0.10–0.44</td>
</tr>
<tr>
<td>P*</td>
<td>0.0258</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0007</td>
<td>0.013</td>
<td></td>
</tr>
</tbody>
</table>

*Pairwise comparisons of two successive age groups (Kolmogorov–Smirnov test).
the Vitros method and the biuret method, taking the latter as reference. Thus, the Hitachi values, which were on average 21% lower than the Vitros values, must be corrected when the present Vitros reference intervals are used to avoid false-negative results.

In conclusion, the pediatric reference intervals reported here complete previous studies in adults and children. Narrow and age-related reference intervals are proposed for an analyzer that is widely used in clinical chemistry and is based on dry chemistry. These proposed intervals may improve the predictive performance of CSF protein assays, particularly in the first 3 months of life.

**Supplemental Information**

Data from this study are available as a supplement from the *Clinical Chemistry* Web site. The file can be accessed by a link from the on-line Table of Contents (http://www.clinchem.org/content/vol46/issue3/).

**References**