Establishment of a Reference Interval for β2-Microglobulin in Cerebrospinal Fluid with Use of Two Commercial Assays
Charles T. Lutz, Steven H. Cornell, and James A. Goeken

Increased concentrations of β2-microglobulin in cerebrospinal fluid have been used to detect central nervous system involvement with metastatic cancer and with neurological complications of AIDS. However, no adequate reference interval study has been reported for β2-microglobulin in cerebrospinal fluid. We established a reference interval with both the Abbott IMx microparticle enzyme-linked immunoassay (EIA) (0.6–2.0 mg/L) and the Pharmacia β2-micro EIA 96 method (0.8–2.2 mg/L) for β2-microglobulin in cerebrospinal fluid. The two methods correlate well, with the latter method giving slightly higher values. β2-Microglobulin increases with age in adults by about 0.1 mg/L every 7.5 years, with no significant difference between genders.

Additional Keyphrases: enzyme immunoassay - age-related effects

Departments of Pathology and Radiology, University of Iowa, Iowa City, IA 52242.
Received June 27, 1990; accepted November 5, 1990.
The concentration of β2m in cerebrospinal fluid (CSF) has been proposed to reflect metastasis of malignant cells to the meninges in various cancers (7–10). Leukemias and lymphomas frequently metastasize to the meninges, posing an important diagnostic problem. Diagnosis is difficult because central nervous system (CNS) relapse may occur early in disease, or even months to years after complete remission is achieved (11). Furthermore, initial symptoms are usually nonspecific—headache, lethargy, nausea (11). The mainstay of diagnosis is detection of malignant cells in the CSF, yet lymphoblasts and myeloblasts are sometimes difficult to identify, and no firm diagnostic criteria are available (11). Thus, any test that helps diagnose CNS metastasis is quite useful in guiding the care of these patients. In several studies, β2m in CSF has been shown to accurately mark meningeal metastasis by leukemias and lymphomas (9, 12–15), and in some cases an increase in β2m in CSF precedes cytological evidence of metastasis (9, 12, 13, 16). In addition, β2m in serum is useful in predicting how soon patients with human immunodeficiency virus (HIV) will develop clinical acquired immunodeficiency syndrome (AIDS) (17, 18); assay of β2m in CSF has been proposed for determining AIDS dementia (19, 20).

In the management of both cancer patients and HIV-infected patients, serial measurements of β2m in CSF are expected to more accurately reflect CNS involvement than an isolated value. However, many clinical situations arise that may require the physician to interpret an isolated value. Thus, it is imperative to establish a reference range with which to compare an isolated value from a patient. Several reference range studies for β2m in CSF have been reported (7–10, 15, 21, 22), but all must be interpreted with caution, owing to small sample size or failure to exclude patients with inflammatory or psychiatric diseases who may have increased β2m in CSF.

Here we compare the Abbott IMx microparticle EIA with the Pharmacia β2m EIA 96 for measuring β2m in CSF, and we report a reference interval for β2m in CSF from 139 adult patients undergoing lumbar puncture for suspected disc disease.

Materials and Methods

Patient selection. Patients (88 men, 51 women; mean age 50.0, range 20–82 years) in the reference interval study underwent lumbar puncture before myelography for back or neck pain. Patients were excluded from the reference interval group if they carried a diagnosis of malignant, inflammatory, or psychiatric disease. A different group of patients received lumbar puncture for suspected malignant or inflammatory disease.

Sample collection and analysis. Nonbloody CSF was collected by puncture of the L2–L3 or L1–L2 intervertebral space. Within 3 h of collection, samples were centrifuged at 500 × g, and stored for up to one year at −20 or 4 °C until assay. β2m values in CSF did not decrease upon extended storage at either temperature (data not shown). We determined β2m values by using either the β2-micro EIA 96 (Pharmacia, Piscataway, NJ) or the IMx (Abbott, N. Chicago, IL) as directed by the manufacturers. The manufacturer's stated limit of sensitivity for the Pharmacia β2-micro EIA 96 assay is 0.8 mg/L, with a reference interval in serum of 0.8–2.6 mg/L. For the Abbott IMx assay, the stated limit of sensitivity is 0.005 mg/L and the reference interval in serum is 0.6–2.0 mg/L.

Data analysis and graphics were performed with Lotus 1-2-3 version 2.0 software. Parameters of skewness and kurtosis were calculated as described by Solberg (23). Reference intervals were determined by percentile estimation as described by Reed et al. (24). For method comparison, we calculated linear regression by the method of Deming (25). Because the manufacturers report nearly equal CVs for β2m in serum, we set the ratio of errors expected for the two methods at 1. For comparison of β2m values with age, we calculated linear regression by the least-squares method.

Results

Test comparison. To compare the Pharmacia β2-micro EIA 96 test (y) with the Abbott IMx microparticle EIA test (x), we determined β2m concentrations in CSF samples from 104 of the patients without malignant, inflammatory, or psychiatric disease. To include higher values in our comparison, we also tested CSF from 38 patients with one or more of those diseases. For all 142 samples, the assays correlate well, with the Pharmacia β2-micro EIA 96 assay producing slightly higher values: y = 1.13(SE 0.16)x + 0.08 mg/L.

Reference interval. By the Abbott IMx assay, CSF from 139 patients (ages 20–82 years) without malignant, inflammatory, or psychiatric disease showed β2m values of 0.6–2.1 mg/L (Figure 1). There were no outliers, as defined by Reed et al. (24); because the distribution was significantly skewed, we used a nonparametric method (24) and estimated a reference interval of 0.6–2.0 mg/L. By using the Pharmacia β2-micro EIA for 104 samples from patients with back or neck pain, the nonparametric reference interval was 0.8–2.2 mg/L.

Effect of age and gender on β2m in CSF. Because a
correlation between log ($\beta_m$ concentration in CSF) and age has been reported (9), we searched for such a correlation in our data (Figure 2). In our population of 139 adult controls, $\beta_m$ in CSF, without ($y$) or with ($y'$) logarithmic transformation, was correlated significantly with age ($x$): $y = 0.0130(SE 0.0017)x + 0.454(SE 0.275) \text{mg/L} (r = 0.55)$, and $y' = 0.00482(SE 0.00064)x - 0.216(SE 0.104)\log \beta_m \text{ (mg/L)} (r = 0.54)$. That is, the expected mean concentration of $\beta_m$ in CSF changes by $\sim 0.1 \text{ mg/L}$ for each 7.5 years difference from the mean age of 50. However, because of the large scatter of values within each age group (as reflected by the correlation coefficient of 0.55), we recommend using our reference interval in routine clinical practice without an age correlation.

In contrast to age, gender does not significantly affect $\beta_m$ concentrations in CSF; even though the women in our reference group were an average of three years older than the men, the CSF $\beta_m$ values were nearly identical ($1.12 \pm 0.36 \text{ mg/L}$ for women, $1.09 \pm 0.30 \text{ mg/L}$ for men).

Discussion

$\beta_m$ in CSF has received increased attention as an analyte in monitoring patients with malignancy or HIV infection. Leukemias and lymphomas frequently metastasize to the CNS; CNS metastasis may cause relatively nonspecific early symptoms. Thus, a laboratory test that increases the index of suspicion for CNS involvement may be useful in this group of patients. The weight of clinical studies shows that $\beta_m$ in CSF is a useful tool for detecting CNS metastasis in patients with lymphomas and leukemias (9, 12-15) and sometimes precedes other laboratory indices (9, 12, 13, 16). However, because $\beta_m$ is a nonspecific marker of inflammation, increased values must be interpreted with caution, especially in the setting of recent CNS irradiation or intrathecal chemotherapy (7, 14-16). Use of this marker may also be limited in acute lymphocytic leukemia of childhood (16, 26).

HIV also has a tendency to spread to the CNS and cause various neurological symptoms that are frequently difficult to diagnose. It has been proposed that an increase of $\beta_m$ in CSF is an indicator of both the presence and severity of AIDS dementia complex (19, 20). As with malignancy, however, the nonspecific nature of the $\beta_m$ marker requires that values be interpreted in a clinical context because CNS infection and lymphoma secondary to HIV immunosuppression will also cause increased $\beta_m$ in CSF (19).

Because of the growing importance of $\beta_m$ in CSF, we established a reference interval for $\beta_m$ in CSF from 139 adult patients without malignant, inflammatory, or psychiatric illness. Our reference interval of 0.6-2.0 mg/L matches the reference interval established by Abbott for $\beta_m$ values in serum. Several reference intervals for $\beta_m$ in CSF from adults have been reported. However, all (7-10, 12-14, 16) except two reports (15, 21) involved <50 individuals in the reference group (Table 1). For example, Twijnstra et al. (9) report a reference range of 0.65-2.2 mg/L, derived from 48 patients. In other studies, the reference population includes patients with solid tumors (12), diffuse neurological symptoms (7, 10, 14, 15), or psychiatric symptoms (10). In one study, 110 control patients were free from cancer and without structural lesions of the CNS, but were not described further (21).

In the current study, all patients in the reference group underwent lumbar puncture for back or neck pain. We excluded from the control group any patients with a diagnosis of inflammatory or autoimmune disease (collagen vascular disease, vasculitis, inflammatory bowel disease, thyroiditis, etc.), cancer, central or peripheral nervous system inflammatory disease, or psychiatric illness. Although not entirely healthy, this population likely reflects normal values.

Because the youngest patient studied was 20 years

<table>
<thead>
<tr>
<th>Population studied</th>
<th>Method</th>
<th>Reference interval, mg/L</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 with nonhematopoietic tumor</td>
<td>RIA</td>
<td>1.5 ± 0.2*</td>
<td>12</td>
</tr>
<tr>
<td>45 adults with headache, dizziness, pain</td>
<td>RIA</td>
<td>1.1 ± 0.3b</td>
<td>7</td>
</tr>
<tr>
<td>23 hospitalized, free of meningeal disease</td>
<td>RIA</td>
<td>0.0-2.0</td>
<td>8</td>
</tr>
<tr>
<td>48 with low back pain</td>
<td>RIA</td>
<td>0.65-2.2</td>
<td>9</td>
</tr>
<tr>
<td>15 healthy controls</td>
<td>RIA</td>
<td>1.30 ± 0.69c</td>
<td>13</td>
</tr>
<tr>
<td>41 with headache and mild psychosis</td>
<td>RIA</td>
<td>≤1.9</td>
<td>10</td>
</tr>
<tr>
<td>33 adults with headache, dizziness, pain</td>
<td>RIA</td>
<td>0.7-1.9d</td>
<td>14</td>
</tr>
<tr>
<td>13 clinically free of CNS disease</td>
<td>EIA</td>
<td>1.58 ± 0.41b</td>
<td>16</td>
</tr>
<tr>
<td>110 without malignancy or structural CNS lesions</td>
<td>RIA</td>
<td>0.65-2.20</td>
<td>21</td>
</tr>
<tr>
<td>153 with neurological disease</td>
<td>RIA</td>
<td>0.99 ± 0.04*</td>
<td>15</td>
</tr>
<tr>
<td>23 &quot;normal&quot; children</td>
<td>RIA</td>
<td>1.1 ± 0.5b</td>
<td>22</td>
</tr>
</tbody>
</table>

* Mean ± SEM. * Mean ± SD. * Error about the mean not specified.
* Approximate values read from a figure.

![Fig. 2. Correlation of CSF $\beta_m$ with age in the 139 control patients used in the reference interval study](image-url)

$\beta_m$ values were determined with the Abbott IMx method. The various arbitrary symbols denote $\beta_m$ values of individual patients. Overlapping symbols denote multiple patients with the same age and $\beta_m$ value.
old, our results do not establish a range for the pediatric population. However, one study (22) of 23 "normal" children <15 years old, found a mean CSF β2m of 1.1 (SD 0.5) μg/mL, with a range of 0.1 to 1.9 μg/mL. This fits well with our mean value of 1.1 μg/mL and our reference range of 0.6–2.0 μg/mL, but is somewhat higher than that predicted by the age adjustment of 0.1 μg/mL for every 7.5 years from age 50.

As for most analytes, serial measurements of β2m in CSF from the same patient are expected to serve as a better negative control than is a reference interval from a "normal" population. However, because physicians increasingly are required to interpret isolated β2m values in CSF, the reference interval reported here may be useful.

We thank Dianne Eggers, Stephanie Balke, and Lisa Horning for technical assistance, and Kelly D. Smith for German–English translation. This work was supported by Pharmacia and Abbott.

References