Significance of Cerebrospinal Fluid sIL-2R Level as a Marker of CNS Involvement in Acute Lymphoblastic Leukemia

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Abstract. Soluble IL-2 receptor (sIL-2R), total protein, uric acid, glucose, aspartate aminotransferase (AST) and lactate dehydrogenase (LD) levels were analyzed in 153 (19 cytology(+), 134 cytology(-)) pairs of CSF and serum samples and the data were compared with the results of cytologic examination to find new CSF markers of CNS involvement in 77 patients with acute lymphoblastic leukemia (ALL). The CSF leukocyte count of cytology(+) samples averaged 107.6±362.4 cells/µl, and was higher than that of cytology(-) samples (1.0±3.4 cells/µl, p = 0.001). The CSF sIL2-R level of cytology(+) samples averaged 162.1±247.7 U/ml, and was higher than that of cytology(-) samples (11.2±44.6 U/ml, p <0.001). The CSF total protein, uric acid, glucose, AST, and LD levels were not significantly different in cytology(+) and cytology(-) samples (p >0.05). ROC curves showed that the discrimination power of CSF sIL2-R for the presence of leukemic blasts was better than that of CSF leukocyte counts. With a cut-off value for CSF sIL2-R at 10 U/ml, the sensitivity was 89.5% and the specificity was 89.6%. With a cut-off value for CSF leukocyte count at 4 cells/µl, the sensitivity and specificity were 47.4% and 63.2%, respectively. In conclusion, CSF sIL2-R level is a valuable marker of CNS involvement in ALL patients; a level of >10 U/ml may serve as an objective indicator of CNS involvement in conjunction with conventional cytology and the CSF leukocyte count.

Keywords: acute lymphoblastic leukemia, CNS relapse, cerebrospinal fluid, sIL-2R

Introduction

Acute lymphoblastic leukemia (ALL) is the most common leukemia in children [1]. Although marrow replacement is a major cause of symptoms in ALL patients, many important syndromes result from extramedullary invasion, particularly central nervous system (CNS) involvement. A more aggressive therapeutic approach is needed after confirmation of CNS involvement in children with ALL, either at diagnosis or during relapse. The intensification of therapy is fundamental for improving the cure rate in these patients, but it is associated with higher risks of immediate and late deleterious effects [2,3]. Therefore, unequivocal diagnosis of leukemic CNS involvement is extremely important for planning the appropriate treatment.

Less than 5% of children with acute leukemia have clinical symptoms related to CNS involvement at the time of diagnosis [4], and the CNS is the first site of relapse in standard risk ALL patients [5]. However, children with detectable lymphoblasts in cerebrospinal fluid (CSF) are generally asymptomatic. Therefore, diagnosing a CNS relapse depends on the laboratory data and cytological examination. Leukemic blasts in the CSF can be identified using cytocentrifugation and Wright-Giemsa staining. Such morphological evaluation is needed to distinguish the pleocytosis of leukemic meningitis from that induced by intrathecal chemotherapeutic agents (arachnoiditis) or from CNS infections [6,7].
Sometimes, the cytologic examination does not allow a clear distinction between lymphoblasts and normal cells, and additional methods for precise identification of leukemic cells in CSF are necessary [8,9]. Some molecular techniques for the diagnosis of CNS involvement by neoplastic cells have been proposed, but the cell counts, protein, and glucose levels in the CSF do not appear to be reliable as indicators of CNS leukemic infiltration [8,9].

This study analyzed chemical components of CSF samples and compared these data with the results of cytological examination to identify new CSF markers of CNS involvement of ALL. Several chemical constituents were selected as potential CSF markers of CNS leukemia, based on the rationale that their levels depend on cellular metabolism and are easy to analyze in clinical laboratories. Such analytes include lactate dehydrogenase (LD), aspartate aminotransferase (AST), uric acid, total protein, glucose, and soluble interleukin-2 receptor (sIL-2R).

sIL-2R is released along with interleukin-2 from activated T cells. The sIL-2R level in the blood reflects immune activity, and is an important marker for leukemogenesis of adult T-cell leukemia/lymphoma [10]. The sIL-2R level in the blood is elevated in patients with malignant lymphoma [11-14] and ALL [15].

The concentrations of the specified analytes were measured simultaneously in paired CSF and blood samples in order to rule out simple diffusion from the blood to the CSF and to identify the CSF markers of CNS involvement in ALL.

Materials and Methods

One hundred and fifty-three pairs of CSF and serum samples (306 samples in total) from 77 ALL patients were selected from the Catholic Human Sample Bank, Seoul, Korea. The patients gave informed written consent to use any specimens that remained after their laboratory tests were completed.

CSF and serum sIL-2R levels were determined using a sandwich enzyme immunoassay method (Cellfree IL-2R Medex, Kyowa Medex Ltd., Tokyo, Japan), and all specimens were tested in duplicate. Briefly, the sIL-2R molecules in the samples or standard controls were bound to polystyrene microtiter wells that had been previously coated with anti-IL-2R monoclonal antibody. Horseradish-peroxidase-conjugated anti-IL-2R bound to the IL-2R antigen captured by the first antibody and completed the sandwich. A substrate was added to the wells after washing to remove the unbound enzyme conjugated antibody. The reaction was stopped by adding H₂SO₄ solution and the absorbance was read at 490 nm. Standard curves were prepared using reference preparations containing known sIL-2R concentrations.

LD, AST, uric acid, total protein, and glucose levels in CSF and serum were measured using an automatic chemistry analyzer (Toshiba TBA-200FR, Tokyo, Japan). The results of cytological examination were obtained from the patients’ medical records.

The level of each parameter is expressed as mean ± SD. Statistical analyses were performed using the SPSS program. Comparisons of each parameter between the cytology(+) (positive) group and cytology(-) (negative) group were performed by the Mann-Whitney U test. Correlations between the CNS and serum levels in each patient were examined by Pearson’s correlation test. P values <0.05 were considered statistically significant. The discrimination power of each marker for the presence of leukemic blasts in the CSF was evaluated using ROC curves.

Results

The number of cytology(+) and cytology(-) CSF samples was 19 and 134, respectively. Table 1 lists the concentrations of various analytes in the CSF and serum specimens from the ALL patients according to the presence of blasts in the CSF. The CSF leukocyte counts of the cytology(+) and cytology(-) CSF samples averaged 108±362 and 1.0±3.4 cells/µl, respectively. The CSF sIL2-R level in the cytology(+) and cytology(-) CSF samples averaged 162±248 and 11±45 U/ml, respectively. The CSF total protein, glucose, uric acid, AST, and LD levels were similar in the cytology(+) and cytology(-) samples (p >0.05).

Serum sIL2-R level averaged 2,987±1,944 U/ml in patients whose paired CSF samples were cytology(+), compared to 822±1,328 U/ml in those with cytology(-) CSF samples (p = 0.003). Serum uric acid level in patients whose paired CSF samples were cytology(+) averaged 6.9±7.1 mg/dl, versus 4.0±1.6 mg/dl in those with paired cytology(-) CSF samples. The CSF uric acid level was approximately 3.7% of the serum level in the ALL patients. The serum total protein, glucose, AST, and LD levels were not significantly different in patients whose paired CSF smple were cytology(+) or cytology(-).

Statistically significant correlations were found between the CSF sIL-2R levels and paired serum IL-2R levels (r = 0.331, p = 0.007) and between the CSF uric acid and paired serum uric acid levels (r = 0.674, p <0.001). The correlations between the total
protein, glucose, LD, and AST levels in the CSF and paired serum specimens were not significant.

ROC curves showed that the discrimination power of CSF sIL2-R levels for the presence of leukemic blasts in the CSF was better than the leukocyte counts of the CSF. At a sIL2-R cut-off level of 10 U/ml, the sensitivity and specificity were 89.5% and 89.6%, respectively (Fig. 1). If the cut-off value was set at 10 U/ml, 17 of 19 cytology positive CSF samples were sIL2-R positive (89.5%), and 120 of 134 cytology negative CSF samples were sIL2-R negative (89.6%). The discrimination power of the CSF leukocyte count was inferior; at a cut-off of 4 cells/µl, the sensitivity and specificity were 47.4% and 63.2%, respectively (Fig. 1).

Discussion

Approximately 15 to 20% of children with ALL have detectable lymphoblasts in the CSF at the time of diagnosis [16,17]. An isolated CNS relapse occurs in <10% of ALL patients who have been treated with regimens including adequate prophylactic CNS therapy [18]. Some children, such as those in whom ALL is diagnosed within the first 12 mo of life, as well as in those with T-cell ALL, have a higher incidence of CNS leukemia at diagnosis [19,20]. Most reports have considered the presence of overt CNS leukemia at diagnosis to be an adverse prognostic indicator [21,22], and such patients, particularly children with ALL, are treated with more aggressive therapy, including radiation therapy [23-28]. Although intrathecal chemotherapy, which is used in conjunction with intensive

Table 1. Concentrations of various analytes in the paired CSF and serum samples of ALL patients according to presence or absence of blasts in the CSF (values are means ± SD).

<table>
<thead>
<tr>
<th>Body fluid sample</th>
<th>Analyte (and units)</th>
<th>CSF Cytology(+) (n = 19)</th>
<th>CSF Cytology(-) (n = 134)</th>
<th>p value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>Leukocyte count (cells/µl)</td>
<td>108±362</td>
<td>1.0±3.4</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>sIL2-R (U/ml)</td>
<td>162±248</td>
<td>11.2±44.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total protein (mg/dl)</td>
<td>31.6±22.2</td>
<td>25.4±20.5</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td>Uric acid (mg/dl)</td>
<td>0.38±1.20</td>
<td>0.17±0.36</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>Glucose (mg/dl)</td>
<td>74±29</td>
<td>75±13</td>
<td>0.818</td>
</tr>
<tr>
<td></td>
<td>Aspartate aminotransferase (IU/L)</td>
<td>13±5</td>
<td>12±9</td>
<td>0.658</td>
</tr>
<tr>
<td></td>
<td>Lactate dehydrogenase (IU/L)</td>
<td>33±16</td>
<td>25±23</td>
<td>0.131</td>
</tr>
<tr>
<td>Serum</td>
<td>sIL2-R (U/ml)</td>
<td>2.987±1.944</td>
<td>822±1,328</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Total protein (g/dl)</td>
<td>6.75±0.29</td>
<td>6.71±0.67</td>
<td>0.914</td>
</tr>
<tr>
<td></td>
<td>Uric acid (mg/dl)</td>
<td>6.85±7.05</td>
<td>3.99±1.58</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Glucose (mg/dl)</td>
<td>127±31</td>
<td>114±30</td>
<td>0.380</td>
</tr>
<tr>
<td></td>
<td>Aspartate aminotransferase (IU/L)</td>
<td>55±36</td>
<td>47±38</td>
<td>0.678</td>
</tr>
<tr>
<td></td>
<td>Lactate dehydrogenase (IU/L)</td>
<td>608±158</td>
<td>573±398</td>
<td>0.866</td>
</tr>
</tbody>
</table>

Fig. 1. Receiver-operator characteristic (ROC) curves of the sIL2-R level and leukocyte count in the CSF for the presence of blasts in the CSF by cytological examination; (sIL2-R; sensitivity 0.895, specificity 0.896 at 10 U/ml; leukocyte count; sensitivity 0.474, specificity 0.632 at 4 cells/µl).
systemic therapy, cranial radiation, or both, is an important component of CNS leukemia treatment, there are many long-term problems associated with CNS treatment and CNS leukemia. Children with a CNS relapse, who receive a second course of CNS treatment or radiation therapy, have a particularly high risk of significant and progressive loss of intellect and growth problems [29,30]. On the other hand, while CNS remission can be successfully induced in >90% of patients with CNS-directed therapy, most patients treated without intensive systemic therapy subsequently develop a bone marrow recurrence [31,32]. Therefore, precise diagnosis of CNS leukemia is very important.

The definition of overt CNS leukemia is controversial and depends on the CSF leukocyte count as well as the presence of leukemic blasts detected by cytological examination of the CSF. Conventional cytology is a useful and reference method for diagnosing CNS involvement. However, its interpretation is difficult in some cases. A viral infection or chemotherapy can transform normal mononuclear cells in the CSF into atypical blast-like cells. In addition, in some cases, malignant lymphoblasts can present as relatively well-differentiated lymphocytes, which reduces the ability of cytomorphology to provide a precise diagnosis [9,33,34]. CSF cytological examination was reported to misclassify the CNS involvement in 4.3% of hemato-oncologic patients [35]. The clinical outcome of patients with <5 leukocytes/µl and with blasts in the CSF at diagnosis is variable, and it illustrates the difficulty in making a diagnosis of CNS leukemia [36-39]. Therefore, an auxiliary method for precise identification of leukemic blasts in the CSF is needed. We tried to find an objective marker to help making a diagnosis of CNS leukemia by chemical analysis of several CSF constituents.

Molecular techniques have been suggested for the diagnosis of CNS involvement by leukemic cells, but they are expensive and difficult to use in all ALL patients. The cell count, protein, and glucose alterations found in the CSF do not appear to be reliable indicators of CNS involvement [9]. In this study, the leukocyte counts of cytology(+) CSF samples averaged 108±362 cells/µl, and were higher than in the cytology(-) CSF samples (1.0±3.4 cells/µl, p = 0.001). However, the ROC curve of the CSF leukocyte count showed that its discrimination power for the presence of leukemic blasts in the CSF was not good; at a cut-off value of 4 cells/µl, the sensitivity and specificity were only 47.4% and 63.2%, respectively. Neither CSF protein or glucose levels proved to be helpful in the diagnosis of CNS involvement by leukemic cells.

The soluble interleukin-2 receptor (sIL-2R) is released from activated T cells along with interleukin-2. In patients with ALL, the sIL-2R level in the serum is significantly higher than in normal people [12,15]. In addition, the sIL-2R level in serum is higher in those patients with a poorer outcome, and is rapidly decreased after initiation of chemotherapy [13,15]. Therefore, CSF sIL-2R level could be increased when the leukemic blasts invade CNS. However, the sIL-2R level in the CSF has not previously been studied in ALL patients.

In the present investigation, the CSF sIL2-R levels of cytology(+) CSF samples were much higher than those of cytology(-) CSF samples. Moreover, the CSF sIL2-R level was not well correlated with the serum IL-2R level; a low CSF sIL2-R level (<10 U/ml) was found in several patients with very high serum sIL2-R level (>3,000 U/ml). This suggests that the CSF sIL2-R originates from the leukemic blasts, rather than from simple diffusion from the blood. Hence, the CSF sIL2-R level appears to be a marker of CNS involvement of ALL.

Interestingly, the ROC curve showed that the discrimination power of the CSF sIL2-R level for the presence of leukemic blasts in the CSF was better than the CSF leukocyte counts. At a cut-off of 10 U/ml of CSF sIL2-R, the sensitivity and specificity were 89.5% and 89.6%, respectively. Quantitative measurements of the CSF sIL2-R are performed using an established, objective analytical method. This suggests that the measurement of CSF sIL2-R is a very useful and objective method for diagnosing CNS involvement of ALL. Although it cannot replace the cytological examination of CSF, it could be a supplementary method for diagnosis of CNS involvement in ALL.

The other putative CSF markers, including uric acid and AST concentrations, did not reflect the presence or absence of leukemic blasts in the CSF. Low correlation was found between CSF uric acid levels and the paired serum uric acid levels (r =
0.674, p <0.001). The average uric acid level in the CSF is known to be approximately 10% of the serum uric acid level [40]. However, the uric acid level in the CSF of ALL patients was approximately 3.7% of the serum level. This suggests that serum uric acid does not diffuse into the CSF equally in all patients. The CSF LD level was reported to be higher in ALL patients than in normal control subjects [34,41]. Neither serum LD nor CSF LD levels showed significant difference between the cytology(+) and cytology(-) groups in this study. However, it is possible that the LD activity in the samples may be artefactiously low because frozen samples were used in the present study.

In conclusion, the CSF sIL2-R level is a valuable marker of CNS involvement in ALL patients and a level of >10 U/ml may be used as an objective indicator of CNS involvement in conjunction with conventional CSF cytology and CSF leukocyte count.

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