

Assesment of Soluble Interleukin-2 Receptor in CSF for the Diagnosis of CNS Disease in Acute Lymphoblastic Leukemia

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ABSTRACT

Background and Aim: Diagnosis of meningeal localization of lymphoid malignancies by means of cytologic examination of the cerebrospinal fluid (CSF) can be difficult. Thus far, no reliable CSF tumor marker has been identified. The aim of this study is to determine prospectively the value of CSF soluble interleukin-2 receptor (sIL-2R) for the diagnosis of CNS leukemic infiltration in acute lymphoblastic leukemia (ALL).

Patients and Methods: CSF soluble IL-2R levels were determined by the sandwich enzyme linked immunosorbent assay in 75 ALL patients. Thirty of the patients had clinical and laboratory evidence of CNS leukemic infiltration; whereas 45 were newly diagnosed cases without evidence of CNS involvement. Routine biochemical CSF tests including microprotein, LDH and glucose level, in addition to CSF leucocytic count were compared to sIL-2R levels in the diagnosis of CNS leukemic infiltration.

Results and Conclusions: CSF sIL-2R was significantly higher in ALL patients with CNS leukemia, with a median sIL-2R of 297 pg/ μ l and a range from 6 to 5840 pg/ μ l, compared with ALL patients without CNS disease as their median sIL-2R was 30 pg/ μ l and ranged from 0 to 129 pg/ μ l (p -value < 0.001). The CSF levels of the sIL-2R were elevated > 100 pg / μ l in 27/30 (90%) of ALL patients with CNS disease; whereas it was only elevated in 3/45 (6.6%) ALL patients without CNS disease. On multivariate analysis, sIL-2R was the most significant diagnostic tool for CNS leukemia. Thus, it can be concluded that sIL-2R can be used as a promising tumor marker in the CSF of ALL patients with CNS leukemic infiltration and can be useful to confirm CNS disease in patients with suspicious cytology.

Key Words: Acute lymphoblastic leukemia (ALL) - CNS leukemia - Soluble interleukin-2 receptors (sIL-2R).

INTRODUCTION

Despite the success of CNS preventive therapy in dramatically reducing the incidence of CNS recurrence in ALL, CNS relapse remains

a significant cause of treatment failure in ALL. CNS relapse is observed in around 10% of ALL patients [1]. In a recent review over the prognostic factors in pediatric ALL, CNS involvement is still considered among the bad prognostic features [2]. It is therefore important to define children with CNS leukemia infiltration as additional therapy usually entails additional morbidity.

Cytologic examination of CSF is the gold standard for establishing the diagnosis of leukemic or lymphomatous meningitis. However, CSF specimens frequently contain only few morphologically recognizable malignant cells [3]. It has been previously concluded that several factors contribute to the difficulty in determining the presence of CNS leukemia including the small number of cells as well as difficulty in morphological interpretation [4]. Unfortunately, false negative results are common [3].

It has been reported that measurements of biochemical markers, soluble factors and immunohistochemistry in CSF are useful in the diagnosis of neoplastic meningitis. Elevated levels of soluble interleukin-2 receptor (sIL-2R; sCD25) in sera have been reported in various hematological malignancies [5]. However, there are few reports [6-8] on the levels of sCD25 in CSF. Therefore, we attempted to investigate if soluble IL-2R in CSF can be used as a diagnostic marker of CNS involvement in ALL.

PATIENTS AND METHODS

Study design: This study was carried out to define the usefulness of sIL-2R estimation in

CSF in the diagnosis of CNS involvement in ALL by studying 2 groups of ALL patients. The first group included ALL patients with clinical and laboratory evidence of CNS infiltration. The second group included newly diagnosed ALL cases with no evidence of CNS infiltration. To diagnose CNS leukemia, the presence of more than 5 mononuclear cells/ μ l in addition to the presence of blasts in CSF were necessary [1].

Patients: The present study was carried out on 75 ALL cases divided into 2 groups, a group of patients having both clinical and laboratory evidence of CNS leukemia (group A) including 30 patients and a group of newly diagnosed cases without CNS disease (group B) including 45 patients. All patients were chosen from the outpatient clinic of the Clinical Pathology Department at NCI, Cairo University during the period from May to November 2001. Diagnosis was established by standard methods including history, clinical examination, peripheral blood, bone marrow examination, cytochemistry, immunophenotyping, lumbar puncture and CSF examination.

Among the newly diagnosed cases ($n = 45$), 36 were children with an age ranging from 18 months to 16 years with a peak age from 2 to 6 years, while 9 were adults with an age ranging from 17 to 75 years with a peak age from 20 to 28 years. As regards the group with CNS disease, 20 cases were children with an age ranging from 2 to 16 years with a peak age from 10 to 14 years, in addition to 10 adult cases with an age ranging from 17 to 55 years and peak age from 40 to 55 years. Among the new cases, there were 35 males (77.8%) and 10 females (22.2%), whereas patients with CNS disease, were 18 males (60%) and 12 females (40%).

Methods of analysis: CSF samples were studied for CSF total leucocytic count using the hemocytometer. The deposit was examined for the presence of blasts after low speed centrifugation for 3 minutes. The supernatant was examined on the same day for microproteins (M-TP), lactate dehydrogenase (LD-P reagent) and glucose using the automatic SYNCHRON CX system and the change in absorbance was directly proportional to the concentration of the compound to be measured in CSF [9]. The remaining CSF was stored at -70°C for further analysis. CSF samples were examined for sIL-2R alpha by a sandwich enzyme immunoassay utilizing

the Quatikine IL-2R Immunoassay kit manufactured by R & D Systems, USA [10]. This kit applies the use of a monoclonal antibody specific for soluble IL-2R alpha.

Statistical analysis: SPSS package (Versions 10.0) was used for data analysis. Mann Whitney test and Non-parametric ANOVA compared medians of two or more than two independent groups. Chi-square / Fischer exact were tests of proportion independence. Pearson correlation analysis aimed at detecting association of quantitative parameters. Logistic regression analysis has been done to depict independent factors that could be associated with CNS relapse. p -value was significant at the 0.05 level [11].

RESULTS

Within group A, 23 patients experienced CNS relapse during maintenance phase of their therapy and 5 during induction therapy; whereas, 2 patients were presenting as new cases by CNS leukemic infiltration. Six of the patients had a bone marrow relapse as well as the CNS relapse. The clinical manifestations of CNS involvement in the group of CNS disease were found to be headache in 23 cases (76%), blurring of vision in 15 (50%), nausea and vomiting in 13 (43%), cranial nerve palsy in 7 (23.4%), irritability, convulsions and disturbed conscious level in 4 (6.6%) and neck rigidity with back pain in one case (3.3%). Only one case within group A did not complain from any CNS manifestations although this case showed numerous blasts in the CSF with increased CSF levels of protein, LDH, sIL-2R and decreased glucose level. Among the newly diagnosed group (group B) only one patient complained from headache at presentation without CNS infiltration, but he developed CNS relapse later during maintenance therapy.

Immunophenotyping results showed that the newly diagnosed group consisted of 34 precursor B cases (87.2%), 4 T-ALL cases (10.2%) and 1 mature B case (2.6%) while among the patients with CNS leukemic infiltration, 13 were precursor B cases (48.1%), 11 T-ALL cases (40.8%) and 3 mature-B cases (11.1%). There was a highly significant higher incidence of T-All subtype than precursor B-types among CNS disease group when compared with the newly diagnosed ALL (p -value = 0.02).

Results of CSF examination are shown in Table (1). Cytological examination of CSF at presentation in the newly diagnosed group revealed 38 cases with no detectable cells, 6 cases showed lymphocytes and one case showed few (< 5 cells) suspicious cells in the CSF without complaining of CNS manifestations and no other abnormalities in the CSF. All cases of CNS relapsed group were necessarily having > 5 blast cells in their CSF. CSF TLC, protein and LDH showed a significant statistical increase in the CNS diseased group ($p < 0.001$). Glucose level of CSF showed significant statistical decrease in the CNS relapsed group (p -value < 0.001).

The sIL-2R level in CSF in the newly diagnosed group ranged from 0 to 129 pg/μl, while its level among the CNS diseased group ranged from 6 to 5840 pg/μl. Median value for sIL-2R was 297 pg/μl for group A and 30 pg/μl for group B. Only 3 cases with CNS relapse had sIL-2R levels in their CSF below 100 pg/μl, two of them were in the third CNS relapse with few blasts. The sIL-2R level in the CSF showed significantly higher levels among CNS relapse group when compared with the newly diagnosed group (p -value < 0.001). The CSF levels of the sIL-2R were elevated > 100 pg/μl in 27/30 (90%) of ALL patients with CNS disease; whereas it was only elevated in 3/45 (6.6%) ALL patients without CNS disease. Fig. (1) shows a scatter of the median values of sIL-2R in the CSF for both studied groups.

There was a moderate positive correlation between the level of CSF sIL-2R in the 75 patients with each of CSF leukocytic count and protein and a negative correlation with glucose ($r = 0.54$, $r = 0.51$ and $r = -0.48$, respectively). These correlations were found to be highly significant with a p -value < 0.001. Table (2) demonstrates the sensitivity and specificity of each test used to diagnose CNS infiltration in ALL cases.

Multivariate analysis: The multivariate analysis was done to evaluate the diagnostic role of the different studied parameters. It was found that sIL-2R was the most significant test followed by LDH and glucose in CSF in the diagnosis of CNS disease (p -values 0.0037, 0.0214 and 0.0424, respectively).

Table (1): Comparison of CSF findings among newly diagnosed and CNS relapse group.

Parameters	CNS relapsed Group (A)	Newly diagnosed Group (B)
TLC, cells/μL	296.66±583.52	5.95±4.52
Protein, mg/dl	108.93±159.24	23.48±9.37
LDH, U/L	132.21±174.30	41.51±15.46
Glucose, mg/dL	43.40±18.53	66.75±14.19
sIL-2R, pg/μl	297 (6 to 5840)	30 (0 to 129)

Values are in mean ± SD except for sIL-2R levels which are expressed by the median value and range.

All parameters were significantly different in both groups with a p -value < 0.001.

Significant p -value < 0.05.

Table (2): The sensitivity and specificity of different tests studied in the CSF of ALL patients.

Variable	TLC	Protein	LDH	Glucose	sIL-2R
Cut-off	11.5 cells/μL	31.5 mg/dL	59 U/L	58 mg/dL	51 pg/μl
Sensitivity %	93.3	83.5	77	73	93.3
Specificity %	89	82	89	74	80

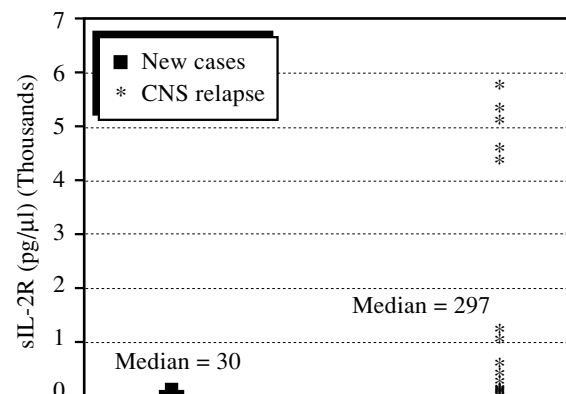


Fig. (1): Scatter of sIL-2R in ALL cases with CNS relapse and new cases without CNS disease.

DISCUSSION

Diagnosis of CNS leukemia has prognostic significance that implies the onset of therapeutic decisions and requires specific therapy with related well known side-effects. The most used widespread diagnostic definition is only based on morphological criteria and despite advances in CSF imaging, cytologic identification of malignant cells in the CSF remains the diagnostic gold standard for leptomeningeal metastases [4]. CSF cytology has a high specificity and false positive results are extremely rare. However,

false negative results are not uncommon. The reported sensitivity of a single CSF cytologic examination ranges from 45 to 94% (mean, 71%). After repeated lumbar punctures, the sensitivity of CSF cytology may increase to 93% [3]. Even lower sensitivity of CSF cytology (38%) may be observed in NHL patients. In some NHL subtypes, the malignant lymphocytes in the CSF cannot be distinguished cytologically from chronic inflammatory cells, thus further limiting the diagnostic utility of cytology in these patients. Therefore, in recent years, some authors have tested soluble factors [6], DNA amplification procedures [2] and flow cytometry [13] to increase the accuracy of the cytological diagnosis.

Interleukin-2 receptor is expressed not only on the surface of activated T or B lymphocytes, but also on certain lymphoid malignancies. The receptor is released from the cell membrane as soluble form. Serum IL-2R is a sensitive and quantitative marker of circulating peripheral blood mononuclear cell activation [14]. Elevated levels of soluble IL-2R in sera have been reported in various hematological malignancies and other conditions associated with active T-cell responses [5]. However, there are few reports concerning the levels of sCD25 in CSF of patients with ALL [6-8].

In a study done by Akl et al. [15] to examine the utility of cytokines and soluble factors in the diagnosis of CNS disease in pediatric ALL cases, it was concluded that the estimation of CSF sIL-2R could be a more promising tool than IL-6 and TNF [15]; however, due to the limited number of cases, more work was needed to focus on sIL-2R as an indicator of CNS leukemia. Therefore, we measured soluble IL-2R in the CSF of ALL patients with a definite meningeal infiltration of leukemic cells and compared it to that of new cases without CNS disease in search for the value of sIL-2R as a diagnostic marker of CNS disease in these patients.

In the present study, soluble IL-2R levels in the CSF of patients with CNS leukemia were significantly higher than those without CNS disease with a median value of 297 pg/ μ l range (6-5840) and 30 pg/ μ l range (0-129) for the 2 groups, respectively. Only 3 patients without CNS leukemia exceeded 100 pg/ μ l. On the other hand, 3 cases within the CNS disease group showed CSF soluble IL-2R below this level, 2

of them were in the third CNS relapse with few blasts. On multivariate analysis, soluble IL-2R levels in the CSF was more significant than CSF leukocyte count and other routine biochemical investigations, i.e. proteins, glucose and LDH, in the diagnosis of CNS leukemic infiltration.

Within the group of CNS leukemia in this study, there was a significantly higher incidence of T-ALL subtype than precursor B-ALL when compared with the newly diagnosed cases without CNS involvement [(11/30 (40.8%) T-ALL versus 4/45 (10.2%) in both groups, respectively)] ($p = 0.02$). T-cell disease is one of the known patient characteristics that are associated with an increased risk of CNS leukemia [1].

Other studies investigating the usefulness of s IL-2R in the diagnosis of CNS disease in hematological malignancies were done in adult T-cell leukemia / lymphoma (ATL) cases [7,8,16, 17] or in lymphoma patients [5] and similar results were obtained. In ATL, leukemia cells are typically CD3+, CD4+, CD8- and mostly CD25+. In the course of meningeal infiltration by these leukemia cells it is possible that sCD4 and sCD25 are released into the CSF from the infiltrating leukemia cells. If so, measurement of these soluble factors in CSF may be useful in diagnosing the meningeal infiltration of ATL cells in patients with ATL as well as indicating the presence of activated T-cells in the CSF in patients with malignant lymphoma [8].

Soluble IL-2R in CSF was found to be markedly elevated in 13/18 adult T-cell leukemia patients with meningeal infiltration [17]. In the latter study, as well as other studies [7,15], it was found that the levels of s IL-2R in CSF are independent of the serum level. This dissociation of the levels of sCD25 in sera and CSF in patients with ATL implies that sCD25 in CSF is not simply diffusion of sCD25 in sera crossing the blood-brain barrier. Levels of sCD25 in CSF are independent of serum levels in patients with neoplastic meningitis including NHL [8]. Considering these results, it is possible that ATL patients clinically diagnosed as having meningeal infiltration without evident CSF pleocytosis and with elevated levels of sCD25 in CSF, in fact had a few infiltrating leukemic cells in their CSF that could not be detected by microscopy [17].

It was found that CSF levels of s IL-2R were significantly higher in patients with ATL & NHL

with meningeal infiltration than in patients with both diseases without meningeal infiltration [7]. In the latter study, the sIL-2R levels in CSF were elevated in 4/4 ATL patients and 3/13 NHL patients with meningeal infiltration [7]. Similarly, 4/9 patients with lymphomatous meningitis (44.4%) and 4/30 patients with carcinomatous meningitis (13.3%) had elevated levels of sCD25 in CSF, but sCD25 in CSF was not associated with specific malignant neoplasms [8]. In the latter study a high percentage of ATL patients with CSF pleocytosis showed extremely elevated levels of sCD25 in CSF. Levels of sCD25 from the ATL patients without CSF pleocytosis was significantly lower than that of ATL patients with CSF pleocytosis ($p < 0.001$) [8].

In the current study, CSF-LDH showed statistically significant higher levels at CNS relapse compared with the newly diagnosed group. Similar reports documented that the detection of elevated LDH in the CSF may be helpful in the evaluation of CNS involvement in patients with hematologic malignancies [16,18]. Moreover, it was concluded that CSF levels of sIL-2R from ATL patients with meningeal infiltration had a tendency to elevate in correlation with numbers of mononuclear cells and LDH in CSF [7].

In our patients with CNS leukemic disease, CSF protein levels were significantly higher in the CNS relapse group compared with the newly diagnosed group. This is in agreement with previous reports [19], but still pleocytosis and higher protein levels without malignant cytologic findings in ALL could be due to a variety of causes e.g infection and during CNS prophylaxis due to development of chemical meningitis [16].

Nowadays, overt meningeal leukemia is uncommon in the foreign practice of ALL management [1]; whereas, overt CNS relapse is still a problem which warrants newer methods for early diagnosis in ALL patients receiving therapy at NCI, Cairo University. In view of the results of this study, we conclude that sIL-2R in the CSF could be used as a diagnostic promising tumor marker in conjunction with determining CSF protein and LDH levels for the diagnosis of ALL patients with leukemic infiltration especially in cases with suspicious cytology.

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