Elevation of Cerebrospinal Fluid Soluble CD27 Levels in Patients With Meningeal Localization of Lymphoid Malignancies


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 markers have been identified. CD27 is a transmembrane disulfide-linked 55-kD homodimer present on most peripheral blood T cells and on a subset of B cells. CD27 is also expressed on human malignant B cells and high levels of soluble CD27 can be present in the serum of patients with B-cell malignancies. The aim of this study is to determine prospectively the diagnostic value of CSF sCD27 as a tumor marker in patients with meningeal localization of lymphoid malignancies. CSF sCD27 levels were determined by sandwich enzyme-linked immunosorbent assay. The optimal cut-off value using receiver operator characteristics curves was found to be 10 U/mL. sCD27 levels were normal in all 50 control patients (lumbar disc protrusion) and in 39 of 40 samples obtained from patients with either solid tumors or acute myeloid leukemia. Of 104 CSF samples from 70 children with acute lymphoblastic leukemia (ALL) or non-Hodgkin’s lymphoma (NHL) undergoing routine central nervous system (CNS) staging, sCD27 was false positive and false negative in only one sample each. In 7 patients with positive CSF studied longitudinally, sCD27 levels correlated very well with remission and relapse. sCD27 levels were not nonspecifically increased by the administration of cytostatic drugs. Finally, sCD27 was also elevated in the 4 patients studied with primary central nervous system lymphoma (PCNSL). CSF sCD27 is a promising tumor marker in patients with either meningeal localization of lymphoid malignancies or PCNSL, and can be useful in the differential diagnosis of CNS involvement by either lymphoid malignancies or solid tumors.

Cytologic examination of the cerebrospinal fluid (CSF) is the most important tool for the diagnosis of meningeal localization of lymphoid malignancies. However, early meningeal involvement may be difficult to detect and false-negative rates up to 40% for the first lumbar puncture have been reported. Furthermore, it is prone to interobserver variability and in some cases it is impossible to differentiate malignant from reactive nonmalignant cells by morphologic criteria alone. The additional value of immunocytochemistry using monoclonal antibodies (MoAbs) is limited because of its low sensitivity. On the other hand humoral CSF tumor markers, such as lactic dehydrogenase (LDH), fibronectin, and tumor necrosis factor α (TNF), have a disappointing specificity. Although CSF β2 microglobulin (β2M) appears to have some diagnostic value, it is consistently increased by the intrathecal administration of cytostatic drugs, which makes it an unsuitable marker during prophylaxis and therapy of meningeal involvement. The use of cleaved L-selectin as a marker for detection of meningeal involvement is restricted to patients with L-selectin+ leukemia (≥60% of all leukemia patients).

CD27 is a transmembrane disulfide-linked 55-kD homodimer present on most peripheral blood (PB) T cells and on a subset of B cells. It is a lymphocyte-specific member of the TNF-receptor family. Activation of T cells via the TCR/CD3 complex results in upregulation of CD27 expression on the cell membrane as well as in the release of a soluble 32-kD form of CD27 (sCD27). sCD27 can be detected in the supernatant of activated lymphocytes and in body fluids like plasma, urine, and CSF. Mildly elevated levels of sCD27 are found in the serum and CSF of patients with a variety of immunopathological conditions such as multiple sclerosis and acquired immunodeficiency syndrome. We recently showed the expression of CD27 on human malignant B cells and showed that very high levels of sCD27 can be present in the serum of patients with B-cell malignancies.

In this study we prospectively investigated the diagnostic value of CSF sCD27 as a tumor marker in patients with meningeal localization of lymphoid malignancies, using cytology combined with clinical follow-up as the ‘golden standard.’ Moreover, the results were compared with β2M levels.
III: 45 patients (144 samples) suspected of meningeal localization of ALL (18: 7 cALL, 2 pre B-ALL, 5 B-ALL, 4 T-ALL) or NHL (27); IV: 4 patients (8 samples) with primary central nervous system lymphoma (PCNSL); V: 32 patients (40 samples) with either myeloid leukemia (8) or solid tumors (24), suspected of meningeal metastases. Solid tumors included 13 breast carcinomas, 2 lung cancers, 2 astrocytomas, 1 medulloblastoma, 1 renal cell carcinoma, 1 germ cell tumor, 1 rhabdomyosarcoma, 1 ovarian cancer, 1 thyroid cancer and 1 unknown primary tumor.

CSF was obtained by lumbar puncture; one portion was processed directly for cytologic examination; the rest was frozen immediately and stored at −20°C. In previous experiments we have shown that repeated freezing and thawing has no effect on sCD27 levels.

If available, paired CSF/serum samples were measured for sCD27 and β2M in patients from group III and V and the CSF/serum ratio was calculated. Clinical data were obtained by review of the medical charts.

Cytological evaluation. Two milliliters of CSF was collected in tubes containing 2 mL of medium composed of RPMI and human albumin. The samples were centrifuged at 340g for 8 minutes. The pellet was resuspended and cytosin slides were stained with Jenner-Giemsa (Jenner: BDM laboratory supplies, Poole, UK; Giemsa: Diagnostica, Darmstradt, Germany). If sufficient material was available, immunocytochemistry was performed with fluorescein-activated cell sorter analysis using MoAbs against CD4, CD8, CD19, CD20, kappa and lambda. During a recent analysis in our laboratory no major discrepancies were found between cytology and immunocytochemistry (van den Berg and Vet et al, manuscript submitted), these data are not shown.

sCD27 enzyme-linked immunosorbent assay (ELISA). sCD27 levels were determined by ELISA using two CD27 MoAbs (CLB-CD27/1 and CLB-CD27/3; both IgG2A; purified from ascites) directed against nonoverlapping epitopes (sandwich ELISA), essentially as described before. The concentration of sCD27 was expressed as units per milliliter, by reference to a standard curve obtained from absorbance values in serial dilutions of pooled plasma from 15 healthy blood donors. The amount of sCD27 in pooled plasma was arbitrarily set at 100 U/mL. The detection limit of this assay is 2 U/mL. In relation to the commercially available sCD27 ELISA kit (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service), the values resulting from the ELISA we used are approximately 1.5 times lower.

The main difference between both ELISAs is that in the CLB-ELISA PB mononuclear cell culture supernatant is used for the standard curve instead of pooled human serum. The correlation between both ELISAs is excellent (r = .98, data not shown).

β2M levels were measured using a standard Abbott MEIA (Abbott Park, IL).

Statistical analysis. For calculation of sensitivity and specificity of the assay only the first CSF sample(s) obtained for diagnosis of meningeal localization were used. Because no definitive 'golden standard' is available, we defined the CSF to be tumor-positive when either cytology was positive, or when cytology was negative but meningeal localization of NHL or ALL (group III). When using a cut-off value of >10 U/mL, only 1 of 104 samples tested was false positive and only 1 was false negative, leading to a sensitivity of 83% and a specificity of 96%. For patients in group III (presenting with clinical signs of meningeal localization of NHL or ALL) the AUC was 0.95. Also in this group a cut-off value of >10 U/mL provided the best discrimination between positive and negative samples with a sensitivity of 100% and a specificity of 82%. Results of the statistical analysis are summarized in Table 1.

In Fig 2 a dot plot of all samples obtained from all diagnostic lumbar punctures is shown. sCD27 levels were normal in all 50 control patients (group I) and in 39 of 40 samples of 32 patients with nonlymphoblastic leukemia or solid tumors (group V). Within this group, 10 of the patients with solid tumors also had cerebral metastases. In contrast, all patients with meningeal and/or cerebral localization of lymphoid malignancies (group III) had increased CSF sCD27 levels (median 41 U/mL; range 11 to 1,288 U/mL). Moreover, in 4 patients with PCNSL, all 4 first and 7 of 8 total samples were positive for sCD27 whereas cytology was negative. The sample that was negative for sCD27 was obtained from a patient after treatment with dexamethasone and radiotherapy. In the pretreatment CSF sample sCD27 was elevated. On a repeat computer tomography scan, complete regression of the lesion was seen. CSF sCD27 therefore discriminates excellently between meningeal and/or cerebral involvement by a lymphoid malignancy or by a solid tumor.

RESULTS

Statistical analysis. Because of an expected difference in prevalence of meningeal involvement, the different patient groups were analyzed separately. In group II (children undergoing routine CNS-staging) CSF sCD27 appeared to be an excellent test as is shown by an area under the curve of the ROC-curve (AUC-ROC) of 0.99 (Fig 1). When using a cut-off value of >10 U/mL, only 1 of 104 samples tested was false positive and only 1 was false negative, leading to a sensitivity of 83% and a specificity of 96%. For patients in group III (presenting with clinical signs of meningeal localization of NHL or ALL) the AUC was 0.95. Also in this group a cut-off value of >10 U/mL provided the best discrimination between positive and negative samples with a sensitivity of 100% and a specificity of 82%. Results of the statistical analysis are summarized in Table 1.
Table 1. Results of Statistical Analysis

<table>
<thead>
<tr>
<th>Group II sCD27 CSF</th>
<th>Group III sCD27 CSF</th>
<th>Group III sCD27 Ratio*</th>
<th>Group III β2M CSF</th>
<th>Group III β2M Ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>104</td>
<td>70</td>
<td>36</td>
<td>54</td>
</tr>
<tr>
<td>No. of positive samples</td>
<td>6</td>
<td>26</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Median (positive†)</td>
<td>133</td>
<td>415</td>
<td>0.20</td>
<td>2.6β</td>
</tr>
<tr>
<td>AUC ROC</td>
<td>0.99</td>
<td>0.95</td>
<td>0.91</td>
<td>0.77</td>
</tr>
<tr>
<td>Cut-off</td>
<td>&gt;10%</td>
<td>&gt;10%</td>
<td>&gt;0.06</td>
<td>&gt;1.6β</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>83%</td>
<td>100%</td>
<td>83%</td>
<td>81%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96%</td>
<td>82%</td>
<td>86%</td>
<td>68%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>83%</td>
<td>76%</td>
<td>77%</td>
<td>56%</td>
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<tr>
<td>Negative predictive value</td>
<td>98%</td>
<td>100%</td>
<td>92%</td>
<td>86%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>98%</td>
<td>89%</td>
<td>87%</td>
<td>72%</td>
</tr>
</tbody>
</table>

*Median sCD27 or β2M level of the tumor-positive CSF samples.
†Median sCD27 or β2M level of the tumor-positive CSF samples.
§sCD27 levels in U/mL.
|| β2M levels in mg/L.

In group III, 8 samples obtained from 6 patients tested false positive. These included 5 samples from 3 patients with PB contamination (>1,000/3 red blood cells; sCD27 levels 12 to 27 U/mL) and high serum sCD27 levels: in these patients the CSF/serum ratio was normal; 1 patient with Sezary’s syndrome and CSF lymphocytosis (20 U/mL); and 1 patient each with cytomegalovirus-infection (63 U/mL) and cerebral toxoplasmosis (21 U/mL). On the other hand, in 13 samples, sCD27 levels were more accurate than cytology (cytology negative [3] or noninterpretable [10]). In 7 patients studied longitudinally, sCD27 levels correlated very well with remission and relapse: in 3 patients who responded well to treatment, sCD27 levels normalized, whereas they remained elevated in 2 nonresponders. In Fig 3A and B, 2 patients are shown who relapsed after an initial response to treatment; also in these patients the sCD27 level was found to be a reliable marker for remission and relapse. Moreover, sCD27 levels appeared to correlate with tumor load, as measured by number of blast cells in the CSF (data not shown). As can also be seen in Fig 3A and B, the intrathecal administration of cytostatic drugs did not have any nonspecific effect on sCD27 levels that can therefore be used to monitor disease activity during intrathecal chemotherapy.

As a diagnostic test, CSF sCD27 was superior to β2M levels and CSF/serum β2M ratio as is shown by a larger AUC ROC (Fig 1; P <.01) and higher sensitivity and specificity (Table 1). For β2M, the serum/CSF ratio provided a slightly better discrimination between positive and negative samples than the CSF value alone (Table 1). However, β2M did not discriminate between lymphoid malignancies and solid tumors, as the β2M ratio was also increased in 9 of 40 samples of patients from group V. For sCD27, calculating
these patients were normal.

Chemotherapy. Both patients died with resistant disease.

the CSF/serum ratio did not improve the AUC and overall accuracy of the test (Table 1).

**DISCUSSION**

Early diagnosis is important for successful treatment of a meningeal localization of lymphoid malignancies. Because the detection of malignant cells by cytologic examination of the CSF can be difficult, there is a need for reliable tumor markers.

We have shown in a previous study that in patients with B-cell malignancies serum levels of sCD27 are consistently increased and correlate with tumor load. Therefore, we investigated whether CSF sCD27 could also be of diagnostic value for meningeal localization of lymphoid malignancies.

CSF sCD27 indeed proved to be an excellent tumor marker both in children with ALL or NHL undergoing routine CNS staging and in patients presenting with clinical signs of meningitis, as is shown by an AUC ROC approximating 1.0 in both cases. In patients presenting with clinical signs of a meningeal localization, the sensitivity of the assay was 100% and the specificity 82%. The few false-positive results were mostly caused by contamination of the CSF sample with PB. In these cases the CSF/serum ratio proved to be normal. Therefore, it is useful to measure paired CSF and serum samples, even though in general the CSF/serum ratio does not improve the accuracy of the test. In addition, CNS infection causing T-cell activation is a possible source of false-positive results, although in general the levels measured in patients with meningeal infiltration by malignant lymphoid cells are much higher than those found in other neurological diseases. In 13 samples sCD27 levels were superior to cytology, mostly because the cytology in these samples was noninterpretable. As all but one of the 40 samples of patients with acute myeloid leukemia or solid tumors contained normal sCD27 levels, sCD27 allows excellent discrimination between lymphoid malignancies on the one hand and nonlymphoid hematologic malignancies and solid tumors on the other hand.

Although the diagnosis of PCNSL requires histologic confirmation, it is not always possible to perform a diagnostic biopsy. In these cases a reliable tumor marker would be helpful, as cytology of the CSF is negative in most patients. Because sCD27 levels were elevated in all 4 patients with PCNSL, and in none of the 10 patients with cerebral metastases of solid tumors, it does indeed seem to be a promising tumor marker for lymphoid malignancies.

With biochemical methods it is impossible to distinguish sCD27 released by activated T cells from sCD27 released by malignant cells. Therefore, theoretically the observed increased CSF sCD27 levels could merely reflect the presence of activated T cells in the CSF. However, there was no correlation between the number of normal lymphocytes in the CSF and the level of sCD27. The increase in CSF sCD27 could also not be attributed to PB contamination or blood-brain barrier dysfunction because there was no correlation between serum and CSF sCD27. In fact, there were individuals with normal serum and increased CSF sCD27 levels and vice versa. CSF sCD27 therefore appears to be derived from the malignant lymphoid cells present in the CNS, although this remains to be proven directly.

In our previous study we found expression of CD27 on the malignant cells in 93% of patients with acute or chronic lymphoid leukemia and in 86% of patients with NHL. Our results show that sCD27 levels are helpful both in the diagnosis and follow-up during treatment of patients with a meningeal localization of lymphoid malignancies. The method is easy and reproducible, and can complement information obtained by morphological analysis. When sCD27 levels are normal, it is unlikely that meningeal infiltration is present. sCD27 is also a promising tumor marker in patients with PCNSL, but this still has to be confirmed in a larger group of patients.

**ACKNOWLEDGMENT**

The authors thank the departments of Neurology and Pediatric Haematology (Dr H. Behrendt, Dr H.v.d. Berg) of the Aca-
demic Medical Centre, and the Departments of Neurology and Clinical Chemistry (Dr H. van Ingen) of the Daniel den Hoed Cancer Centre for collection of the CSF samples; the Departments of Hematology and Pathology of the Academic Medical Centre and DDH Cancer Centre (Dr W. Kuenen-Bouwmeester) for cytologic analysis; Mw C. Zumpolle for technical assistance; Dr F.J. Hoek for measurement of β2microglobulin levels; and Dr M.H. Prins for statistical advice.

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Elevation of cerebrospinal fluid soluble CD27 levels in patients with meningeal localization of lymphoid malignancies [published erratum appears in Blood 1996 Oct 1;88(7):2818]

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