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Cerebrospinal Fluid Beta$_2$-Microglobulin: a Study in Controls and Patients with Metastatic and Non-Metastatic Neurological Diseases

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Abstract—*We studied cerebrospinal fluid Beta$_2$-microglobulin (CSF B_2-m) in 197 patients with a variety of neurological diseases to evaluate the usefulness of B_2-m in the detection of meningeal dissemination of malignancy. In the control group we found a relationship between CSF log B_2-m and age ($P < 10^{-4}$). Age standardized reference values were established as 0.65–2.2 mg/l. The results show that CSF B_2-m was elevated in leptomeningeal metastases from solid and haematological tumors. We observed slight elevations of CSF B_2-m in epidural and parenchym metastases from solid tumors. Our study shows that B_2-m in CSF is a sensitive marker for meningeal metastases especially from hemopoietic tumors.*

INTRODUCTION

BETA$_2$-MICROGLOBULIN (B_2-m) has been isolated and characterized from human urine by Berggärd and Bearn in 1968 [1, 2]. It is a small subunit of the Human Leucocyte Antigens (H.L.A.) on the surface of nucleated cells. B_2-m is dissociated from H.L.A. heavy chain and released in free form in extracellular fluid. Lymphocytes and macrophages are particularly rich in B_2-m. B_2-m is present in various biological fluids, such as serum, urine, amniotic fluid, ascites, and cerebrospinal fluid (CSF) [3–6].

In blood B_2-m is removed by glomerular filtration. The serum concentration may increase in various circumstances such as in newborns, in elderly, in renal disorders, in malignancies, in auto-immune and inflammatory disorders [7].

The multiple factors that affect B_2-m concentration in the serum, make it questionable in diagnostic procedures [8]. Raised serum B_2-m values nevertheless are reported in serum of patients with multiple mycloma, non-Hodgkin lymphoma, Hodgkin's disease and chronic lymphatic leukemia [9].

Measurement of CSF B_2-m seems more promising in diagnosis of CNS involvement by haematological tumors [5]. Recently it has been reported that CSF B_2-m increased before the CSF was positive for tumor cells [9, 10]. This may lead to earlier and more specific diagnosis of CNS leukemia and lymphoma. As little data are available concerning:

(1) CSF B_2-m values in normal subjects;
(2) CSF B_2-m values in metastatic and non-metastatic diseases;
(3) the sensitivity and specificity of CSF B_2-m, the present study was set up to further investigate this possible application of the assay.

Without knowledge of the CSF B_2-m from patients with neoplastic and non-neoplastic neurological diseases, CNS involvement by solid and haematological tumors cannot be diagnosed with reasonable confidence. Other investigators reported high level CSF B_2-m in bacterial meningitis [5, 10]. Because this infiltration can be diagnosed by culture, bacterial meningitis was excluded from this study.

MATERIALS AND METHODS

We collected CSF samples from 197 patients with normal renal function. The CSF specimen were coded and stored at −20°C until analyzed by radioimmunoassay. We performed lumbar puncture as a part of clinical diagnostic procedures, and never for the measurement of B_2-m only. CSF

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examinations included protein concentration, glucose content, LDH, culture and cytology with the cytocentrifuge technique. We compared the CSF B_2-m level in 10 patient groups (Table 1). The 48 patients with low back pain with or without leg pain served as a control group. They were otherwise healthy. Myelography was normal in 36 of them and showed a lumbar disc herniation in 12 subjects. Age and sex distributions of the controls and patients are given in Table 1. Patients with malignancies were admitted to the Antoni van Leeuwenhoekhuis during 1981–1983, and patients without malignant diseases to the Department of Neurology of the Slotervaart Hospital during 1981–1982. The criteria used for the diagnosis of leptomeningeal metastases was the presence of tumor cells in the CSF. In 12 patients B_2-m levels were measured in serum as well as in CSF. We determined total protein and lactate dehydrogenase (LDH) in cerebrospinal fluid using the test methodologies of the ACA (Dupont Company, Clinical Systems Division, Wilmington, DE 19898, U.S.A.).

LDH methodology was calibrated to give values similar to results obtained with the method of the German Society for Clinical Chemistry [11]. Glucose was determined using a Beckman glucose analyzer (Beckman Instruments Inc., Fullerton, CA 92634, U.S.A.).

We measured both serum and CSF B_2-m by radioimmunoassay using the Phadebas B_2-m microtest (Pharmacia Diagnostics, Uppsala, Sweden). Briefly, aliquots of 200-fold diluted serum and CSF were incubated for 3 hr at room temperature with ^{125}–I labeled B_2-m and anti–B_2-m antibodies coupled to sephadex particles. Thereafter, the bound and free B_2-m were separated by centrifugation. The radio activity bound to sedimented sephadex particles was then measured. This radioactive uptake varied inversely with the quantity unlabeled B_2-m present.

From the results obtained in the controls, we calculated reference values for CSF B_2-m (Table 1).

STATISTICS

From the results of a preliminary analysis, it was decided to transform the B_2-m logarithmically prior to the final analysis in order to get a closer approximation of the variation of observed values by the normal distribution in all groups. Ordinary linear regression analysis was used to determine the relationship between log B_2-m and sex and age. To test the statistical distribution of values for normality the test of Shapiro and Wilk was used [11].

Skewness indicates that data distribution was not symmetrical. Kurtosis indicates the degree of steepness of the middle part of the data distribution. Kurtosis measures the concentration round the mean value.

RESULTS

We showed the mean and S.D. of CSF B_2-m, S.D., skewness and kurtosis (Table 1). The skewness and kurtosis indicate measurements for the shape of the distribution. In the control group a relationship was found between CSF log B_2-m and age ($P < 10^{-4}$) (Fig. 1). We found no difference between females and males with respect to the slope of log B_2-m against age (P=45) nor with respect to the intercept (P=0.91).

Because of the age dependence of log B_2-m, age standardization is needed in comparing the different groups. From the regression equation in the control group we defined the following quantity as

Table 1. *CSF Beta$_2$-micro microglobulin levels* (mg/l) *in groups of control subjects and patients*

Group	n	Age Mean	S.D.	Male/ Female	$\bar{x}$ (mg/l)	S.D.	Skewness	Kurtosis
Low back pain (controls)	48	48	16	34/14	1.33	0.48	0.77	1.17
Trauma capitis	4	38	26	3/1	1.85	1.12	1.11	−0.70
Neuropathy	6	52	18	5/1	1.56	0.55	0.98	−0.41
Cerebrovascular accident	29	71	12	17/12	1.74	0.48	−0.21	−0.28
Epidural metastases	19	58	11	1/18	1.85	0.97	1.38	1.39
Brain metastases	26	52	17	11/15	1.49	0.67	2.69	8.55
Leptomeningeal metastases solid tumors	25	53	11	6/19	3.22	1.79	0.93	0.55
Leptomeningeal metastases haematologic tumors	9	49	17	7/2	4.42	2.13	0.45	−1.16
Solid tumors without CNS metastases	23	55	16	3/20	1.57	0.70	2.47	5.92
Haematologic tumors without CNS metastases	8	46	22	5/2	1.43	0.86	1.61	1.37

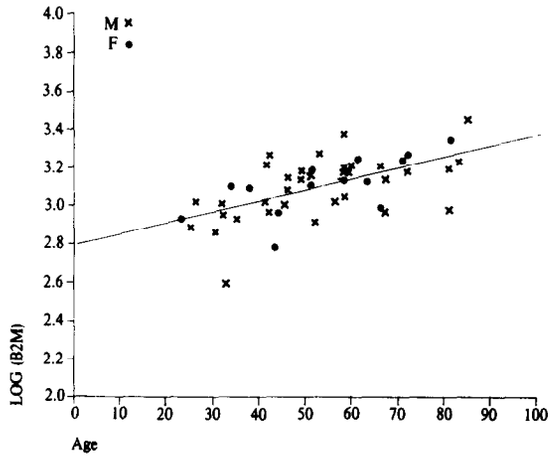

Figure 1. The relationship between log B_2-min in CSF and age of the 48 control subjects. Log B_2-m levels increased significantly with age for the overall group (P=0.0004) independent of sex difference (P=0.86). Regression equation: log B_2-m = 3.081–0.00595 (age: 50).

standardized log B_2-m at 50 yr:

$$-\log (B_2\text{-m})_{AS} = \log (B_2\text{-m}) - 0.00595\ (\text{age} - 50).$$

We found no deviation from normal in groups at a level of $P < 0.10$. Deviation from normal occurred in the patient groups with CVA ($P < 0.05$), brain metastases ($P < 0.02$) and solid tumors without metastases ($P < 0.01$). We defined the reference range of B_2-m standardized at the age of 50 as 0.65–2.20 mg/l. Other investigators described similarly reference ranges [2, 10]. From Table 2 it can be seen that B_2-m used as the sole diagnostic tool leads to a sensitivity of 70% (95% confidence; 47–87% interval) for leptomeningeal metastases from solid tumors and of 90% (52–99%) for leptomeningeal metastases from haematological tumors. Adding all groups with leptomeningeal metastases together we would find a specificity of 93% (87–96%). Counting only the tumor group without leptomeningeal involvement would lead to a specificity of 87% (76–94%). All four patients with spinal epidural metastases and raised CSF B_2-m showed a gross elevation of CSF protein and a complete block by myelography, so clinically seeding was suspected. Of the two patients with intraparenchymal metastases and elevation of CSF B_2-m levels, the metastases were on CT-scans adjacent to meningeal surface, but we did not find tumor cells in the CSF.

The serum B_2-m and CSF B_2-m levels did not correlate in 12 patients of the different groups.

DISCUSSION

In recent years many investigators frequently diagnosed leptomeningeal tumors [12–14]. This increase has been generally attributed to longer survival of patients because of more effective drug treatment of the primary tumor and the failure of the currently used chemotherapeutic agents to cross the blood–brain barrier.

Early diagnosis of meningeal metastases is a common diagnostic problem [12, 15, 16]. The false negative rate varies from 5 to 40% of the patients with leptomeningeal tumors. The yield of positive cytology may be enhanced by examination of successive CSF samples [6, 17]. We found raised CSF B_2-m in two autopsy proven cases with lep-

Table 2. Beta$_2$-microglobulin in cerebrospinal fluid. Elevated values in different groups

Group	Total	B_2-m elevated	Total % elevated
Control subjects	48	0	0
Trauma capitis	4	1	25
Neuropathy	6	1	17
Cerebrovascular accident	29	0	0
Epidural metastases	19	4	21
Brain metastases	26	2	8
Leptomeningeal metastases solid tumors	25	17	68
Leptomeningeal metastases haematologic tumors	9	8	89
Solid tumors without CNS metastases	23	2	9
Haematologic tumors without CNS metastases	8	2	25
Totals	197	37	

tomeningeal tumors, with negative cytological findings in CSF. Tumor-associated proteins in CSF, the so-called tumor markers, for the early identification of metastases to the meninges, have been described previously (6, 9, 10, 18–20). From these studies insufficient data are available on patients with parenchymal and epidural metastases and non-neoplastic neurological diseases.

Such information is necessary to evaluate sensitivity and specificity of the B_2-m assay in CSF for the diagnosis of leptomeningeal tumors [21, 22]. In our control group CSF B_2-m increases significantly with increasing age. This complicates the comparison of different groups of diseases. To overcome this difficulty we standardized CSF B_2-m values for age using the regression equation found in the control group.

Like Schaub *et al.* we have not observed any correlation between corresponding serum and CSF B_2-m levels in a group of neoplastic patients, which suggests an autonomous production of B_2-m in the two compartments [23]. Both Mavligit *et al.* [9] and Koch *et al.* [10] recently published results of a study about CSF B_2-m in CNS metastasis. They did not mention an effect of age on CSF B_2-m. However, we found CSF B_2-m levels increased significantly with age for the overall group ($P < 10^{-4}$). An additional finding was that B_2-m as a sole diagnostic tool leads to a sensitivity of 70% for meningeal carcinomatosis and of 90% for meningeal dissemination of haematological tumors [21, 22].

A specificity of 93% was calculated adding all patients together with non-malignant and malignant neurological diseases but without malignant meningeal dissemination. We detected a raised B_2-m level in two patients with cerebral and four with epidural metastasis. These elevations might be owing to the invasion of submeningeal or subependymal parenchym, so that malignant cells may not be detected. On CAT-scan we noted subependymal brain metastasis in two patients.

From the present study we conclude that the determination of age standardized B_2-m in CSF affords a valuable tool in the diagnosis for leptomeningeal tumor, more appropriate for lymphoma than for solid tumors.

Further studies will be needed to determine the validity of the test in monitoring progression of the disease and evaluating tumor response to therapy.

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