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Unreliability of β-2-Microglobulin in Early Detection of Central Nervous System Relapse in Acute Lymphoblastic Leukemia

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Abstract—*The value of serial determination of the cerebrospinal fluid (CSF) β-2-microglobulin (β_2m) level for early detection of acute lymphoblastic leukemia (ALL) in the central nervous system (CNS) has been prospectively studied in 30 children. β_2m was determined by micro-ELISA assay. Results demonstrated a sensitivity of 40% (95% confidence interval, 5.3–85.3%) and a predictive value of 8% (95% confidence interval, 1.0–26.0%). In post-irradiation syndrome, as well as in viral infection with cytopathological changes of white cells in CSF, β_2m values proved to be significantly higher than in incipient CNS relapse, and these conditions, or an unknown cause, are more often associated with β_2m elevation than CNS relapse. No relation was found between CSF white cell counts and β_2m levels. It is concluded that β_2m is* not *an appropriate test for early detection of CNS involvement in children with acute lymphoblastic leukemia.*

INTRODUCTION

IN ACUTE lymphoblastic leukemia (ALL) central nervous system (CNS) relapse is one of the major therapeutic problems. Screening for CNS leukemia is performed by periodical sampling of cerebrospinal fluid (CSF) for cell count, cytopathological investigation and determination of protein level. Early detection of CNS relapse might improve the results of therapy. In the past years reports have indicated that in patients with acute leukemia or lymphoma with CNS involvement CSF levels of beta-2-microglobulin (β_2m) were elevated [1]. Recent studies support these findings [2]. Mavligit and co-workers [1] stated that β_2m levels in CSF and the CSF/serum ratio were well correlated with CNS involvement in patients with acute leukemia or lymphoma. Consequently, they concluded that serial and simultaneous determination of β_2m in serum and CSF might be useful in the early diagnosis of CNS involvement and in the monitoring of intrathecal therapy in patients with acute leukemia or lymphoma.

The aim of the present study is to investigate the value of β_2m determination as a test for early detection of CNS involvement in childhood ALL.

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MATERIALS AND METHODS

Patients

Blood serum and CSF samples were collected at regular intervals during the period June 1981 to August 1982 from all children with ALL ($n = 30$) who underwent lumbar puncture at the Department of Pediatrics of the Free University Hospital. The patients were treated according to the protocols of the Dutch Childhood Leukemia Study Group. The frequency of CSF-β_2m determination depended on the frequency of routine sampling according to the treatment protocol (every 14 weeks), and on the presence of clinical indications to perform a lumbar puncture. CSF was obtained by lumbar puncture (26 patients), or from an Ommaya reservoir (four patients).

The minimal follow-up period was 12 months. Diagnosis of CNS-leukemia was based on the presence of malignant lymphoblasts in cytopathological examination of two samples of CSF taken with an interval of 2–7 days.

Methods

β_2m was determined by a micro-ELISA assay. The test as used in our study is the test described by Ferrua *et al.* [3], using commercially available antibodies.

The wells of the microtitration plate were coated with 200 μl of diluted β_2m antibodies and left overnight at 37°C. The plate was emptied by inversion and washed three times with 220 μl wash buffer in each well. Between washes the buffer was left for at least 5 min in the plate, reducing non-specific adsorption. The wells were filled with 100 μl of β_2m standard or samples, if necessary diluted in phosphate-buffered saline (PBS) containing 0.3% bovine serum albumin (BSA), followed by incubation for 75 min at 37°C. After another washing step, 190 μl substrate solution was added and the plate was incubated at room temperature, in the dark, for 30 min. The enzyme reaction was stopped by the addition of 45 μl of 4 N HCl and the intensity of the color developed was read at 492 nm.

Materials

Rabbit β_2m antibodies (DAKO-Immunochemicals, Copenhagen, Denmark, code A 072), diluted 1/2000 with coating buffer (0.1 M $NaHCO_3$, pH 9.6). Solid phase: 96-well flat bottom microtiter plate, Nunc, No. 4-42404.

β_2m standard was from the Phadebas β_2m-microtest (kindly donated by Pharmacia Diagnostics, Uppsala, Sweden).

Rabbit β_2m-antibodies were conjugated with horse radish peroxidase (DAKO-Immunochemicals, code P 174) and diluted 1/2000 in PBS (pH 7.4: 1.22 g K_2HPO_4, 8.77 g NaCl in 800 ml distilled water, adjusted to pH 7.4 with 4 M NaOH and made up to 1 l).

The wash buffer was 0.3% (v/v) BSA (Poviet, Organon Teknika. Oss, Holland) and 0.1% (v/v) Tween-20 in PBS.

The substrate solution was made up of 20 mg 1,2-phenylene diamine (OPD) and 0.15 ml 30% H_2O_2 in 20 ml citric acid–phosphate buffer (0.1 M citric acid adjusted to pH 5.3 with 0.2 M Na_2HPO_4).

The intensity of the color developed after incubation was measured with an automatic micro-ELISA reader system (Organon Teknika, Oss, Holland).

The results obtained for serum and urine samples of healthy control persons compare well with those described by Ferrua *et al.* [3]. Moreover, a good correlation was found comparing our test with the Behringwerke test for determination of human β_2m (Enzygnost). In 12 sera a correlation coefficient of $r = 0.95$ and in 18 urines of $r = 1.00$ was found. The concentration of serum β_2m in healthy control subjects, as measured with our test system, all fell within the normal distribution as described by Teasdale *et al.* [4], analysing the sera with the Pharmacia Phadebas [^{121}I]β-2-microglobulin test. The within-assay variation of our test was 2% ($n = 20$) and the day-to-day variation was 7.5% ($n = 16$).

Normal values

Normal values of CSF-β_2m reported in previous studies are 1150 ± 370 ng/ml (mean ± S.D.) and 1100 ± 500 ng/ml [5, 6]; ranges have an upper limit of 2000 ng/ml [2, 7].

Reported levels in patients with leukemia and lymphoma without CNS involvement are 2400 ± 300 ng/ml and 1400 ± 660 ng/ml [1, 2]; Deméocq mentions a lower level: 980 ± 460 ng/ml in patients without or with only minor CNS involvement [8].

Regarding the CSF/serum ratio, Tenhunen *et al.* reported a level of 0.79 ± 0.32 [5]; Mavligit *et al.* used a limit of limit of 1.0 [1].

In the absence of established limits for the normal range, we selected criteria for the elevation of CSF-β_2m. As suggested by Mavligit *et al.* [1], we took into account the CSF level itself, the trend in it and the CSF/serum ratio (Table 1).

RESULTS

One hundred and twenty-four paired (blood and CSF) samples were obtained and 30 single samples. The median lumbar CSF-β_2m level was 1050 ng/ml (range, 300–4850 ng/ml); the median level in ventricular fluid was 520 ng/ml (range, 115–2100 ng/ml). The median serum β_2m level was 1450 ng/ml (range, 610–4000 ng/ml).

Sensitivity

During the investigation period five patients showed a new CNS involvement. Data of these patients are listed in Table 2.

In three of the patients with new CNS involvement CSF-β_2m was not elevated according to our criteria (patients, A, B, and C). One other patient (D) had a slight elevation, according to just one criterion (CSF/serum ratio 1.06). The CSF-β_2m value in this patient was rather low (750 ng/ml); there was no trend to elevation either. The fifth patient (E) had a clearly elevated CSF-β_2m. This patient, however, appeared to

Table 1. Criteria for elevation of CSF-β_2m

(a)	CSF-β_2m $\geqslant$ 1500 ng/ml	and/or
(b)	Elevation of CSF-β_2m $\geqslant$ 500 ng/ml compared with the previous determination	and/or
(c)	Decline of CSF-β_2m $\geqslant$ 500 ng/ml at the following determination	and/or
(d)	CSF/serum ratio of β_2m > 1	

Table 2. β_2m levels in CSF in patients with new, untreated CNS involvement

Patient	β_2m in CSF (ng/ml) At time of CNS relapse	Previous level (interval in weeks)	Next level (interval in weeks)	CSF/serum ratio
A*	420	425 (7)	450 (3)	0.29
B†	1100	—	1100 (2)	0.81
C‡	460	700 (14)	820 (2)	0.44
D†	750	750 (14)	540 (1½)	1.06‖
E§	1350‖	—	575 (1)	0.48

*Isolated CNS relapse; CSF from Ommaya reservoir.
†Isolated CNS relapse.
‡Relapse in bone marrow and CNS simultaneously.
§Initial ALL in bone marrow and CNS; after viral meningitis.
‖The elevated β_2m levels are underlined. [Patient D: CSF/serum ratio > 1; patient E: decline ≥ 500 ng/ml at the next determination (see Table 1).]

have contracted a viral meningitis 2 months earlier, which might have been the cause of the β_2m elevation [7]. Thus in this small patient group the sensitivity did not exceed 40% (95% confidence interval, 5.3–85.3%).

Predictive value of positive test

All cases of elevated CSF-β_2m elevation are listed in Table 3. It is clear that CSF-β_2m elevation is associated with new CNS involvement only in a small minority of cases. In this patient group the predictive value of a positive test is 8% (2/25; the four patients already treated for previously diagnosed CNS involvement were left out). The 95% confidence interval is 1.0–26.0%.

Table 3 shows that in our patients β_2m elevation was associated with various conditions. The most important of these were post-irradiation syndrome and viral infection.

Post-irradiation syndrome. During the investigation period six patients received a 25-Gy CNS irradiation within 2–3 weeks as a part of CNS prophylaxis according to the protocol. In all of these patients the CSF-β_2m level was elevated during the period from 9 to 19 weeks after the start of the irradiation. During this period in all cases β_2m was higher than the last level measured before and the first level measured after this period. The difference is statistically significant (signed rank test, $P < 0.05$).

All but one of these patients showed the clinical features of the post-irradiation syndrome (somnolence, malaise, fever) during the same period.

Viral infection. Six patients showed cytopathological changes in CSF cells during a minor general or respiratory infection of apparently viral origin. Clinically there were no signs of meningeal irritation.

Four of these patients had a mild pleocytosis consisting of mononuclear cells (range, 6–31/mm³). In cytological examination these cells appeared to be for the most part activated lymphocytes, but sometimes resembled malignant lymphoblasts. All six patients had elevated CSF-β_2m, which returned to normal without specific treatment within a few weeks, together with the disappearance of pleocytosis and abnormal mononuclear cells.

During a follow-up period of 1 yr none of these patients developed a CNS-relapse of ALL.

Further statistical analysis

CSF levels of β_2m and CSF/serum ratios in the patient groups with post-irradiation syndrome, viral infection with cytopathological changes of

Table 3. Numbers of patients with an elevated CSF-β_2m level

Post-irradiation syndrome	6
Viral infection with cytopathological changes in CSF	6
CNS leukemia, during treatment	4
During CNS chemoprophylaxis	2
New, untreated CNS leukemia	2
After a general infection	1
Cerebral toxoplasmosis	1
Cause unknown	7
Total	29

Table 4. CSF level and CSF/serum ration of β_2m in patient groups with post-irradiation syndrome (PIRS), viral infection with cytopathological changes of CSF-cells (VIR) and with untreated isolated CNS leukemia (CNS-ALL) (mean ± S.D.)

	CSF-β_2m (ng/ml)	CSF/serum ratio of β_2m
PIRS	2825 ± 582.6	2.06 ± 0.220
VIR	1840 ± 878.6	1.19 ± 0.076
CNS-ALL	682 ± 314.8	0.67 ± 0.366

CSF cells and untreated isolated CNS relapse of ALL are listed in Table 4.

The β_2m level in CSF and the CSF/serum ratio proved to be significantly lower in untreated isolated CNS-ALL then in both post-irradiation syndrome and viral infection with CSF-cell changes (Wilcoxon's two-sample test, $P < 0.05$). Furthermore, the CSF/serum ratio was significantly higher in the post-irradiation syndrome than in viral infection ($P < 0.05$).

There was no evidence of a correlation between CSF white cell count and CSF-β_2m level (correlation coefficient $r = -0.021$).

Ommaya reservoir

Ventricular fluid might have a lower level of β_2m than CSF obtained by lumbar puncture. In one patient we simultaneously determined a ventricular β_2m level of 440 ng/ml and a lumbar level of 820 ng/ml. Of the five patients who developed a CNS relapse during the investigation period only one had an Ommaya reservoir at that time. In this patient the CSF-β_2m level showed no change at the moment of CNS relapse. Despite the absence of normal values of CSF-β_2m in ventricular fluid, we can assume that in this patient CSF-β_2m could not have been helpful in detecting CNS relapse.

DISCUSSION

Methods

Our results seem to differ markedly from those of other authors [1, 2]. Theoretically, the method of determination of β_2m could be responsible for that difference (micro-ELISA vs RIA assay). We think we have standardized and documented our determination method sufficiently to rule out this possibility. Until sufficient normal values are available the use of ventricular fluid for β_2m sampling should be avoided. Reference ranges established for lumbar CSF should not be applied to ventricular specimens. Consequently until now only the *trend* in CSF-β_2m can give information in these patients. As indicated before, this problem did not influence the results of this study.

Normal values

A test for detection of CNS leukemia should be a sensitive one. Hence the upper limits of normal chosen here are relatively low (Table 1). Choosing other limits (e.g. CSF-β_2m $\geqslant$ 2000 ng/ml) would not have changed the results substantially, though.

The use of the CSF/serum ratio, as indicated by Mavligit *et al.* [1], can be criticized because no evidence has been found of a relation between both levels [2, 7]. If the CSF/serum ratio as a criterion is abandoned the results of this study are only marginally influenced: in only 4/29 patients with elevation of β_2m (Table 3) was the elevation based merely on the CSF/serum ratio. Of these four patients one had a CNS relapse; the other three belonged to the group 'cause unknown'.

β_2m and CNS relapse

The present study is the first on this subject which is confined to children with ALL. Previous studies have described mixed patient groups [1, 2, 8].

The results of this investigation demonstrate that serial determination of CSF-β_2m and/or the CSF/serum β_2m ratio cannot be regarded as useful tests for early detection of CNS involvement in childhood ALL, as was suggested by Mavligit and co-workers [1].

The sensitivity of the test in this population of patients with *incipient* CNS involvement proved to be low. Previous reports did not record the sensitivity [1, 2, 8]. Moreover, in these investigations the populations studied contained many patients with advanced stages of disease.

In our opinion a prospective study, like the present one, in a population with no known CNS involvement is the best way to determine the utility of a test for early detection of CNS disease [9].

Because of the wide confidence interval (5.3–85.3%) the sensitivity of CSF-β_2m in beginning CNS-ALL might eventually prove to be more favorable than the 40% found in this study. However, even if that proves to be true, the utility of the test remains low because of the low predictive value of a positive result (8%).

This low predictive value is a striking finding. In the past years some patients have received intensive intrathecal chemotherapy based on the mere elevation of CSF-β_2m [1, 10]. Our results show that such a decision is not justified. Treatment should not be based on a test before the reliability of that test is evaluated in the population in which it is intended to use the test.

The number of patients in this study is too low to draw conclusions regarding the value of β_2m as a parameter for the degree of CNS involvement or for monitoring the therapy.

Post-irradiation syndrome

The finding that all patients who received a CNS irradiation during the investigation period demonstrated a substantial rise in CSF-β_2m is in accordance with the reports of Deméocq *et al.* [8, 11], who have recorded a β_2m elevation 4–10 weeks after termination of CNS prophylaxis including cranial irradiation.

Viral infection

Six patients showed cytopathological changes in CSF cells during a viral infection. In this patient group the β_2m level in CSF was significantly higher than in untreated CNS leukemia.

It is known that lymphocytes in CSF during a viral infection may mimic leukemic blast cells. This could lead to a false diagnosis of CNS leukemia [12], and might burden the patient with a long, heavy, dangerous and unnecessary treatment.

Because β_2m may be elevated both in viral infection and in CNS relapse it is not very helpful in distinguishing between both conditions.

Conclusion

We conclude that β-2-microglobulin is *not* an appropriate test for the early detection of CNS involvement in children with acute lymphoblastic leukemia because of a low predictive value and a low sensitivity.

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