Serum $\beta_2$-microglobulin levels at diagnosis and during antitumour treatment in children with malignant neoplasms

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Objective. To assess whether serum $\beta_2$-Microglobulin ($\beta_2$-M) levels might serve as a marker for both diagnostics and treatment monitoring in children with cancer.

Materials and methods. Serum $\beta_2$-M levels and rates of $\beta_2$-M elevated values were estimated by ELISA in 100 children with acute leukaemia and lymphomas ($n=64$) and with malignant solid tumours ($n=36$). The study was performed prospectively, the analyses were performed before treatment, in partial and complete remission, after therapy and during relapse or progression of cancer. The control group consisted of 30 healthy children.

Results. Median pre-treatment $\beta_2$-M levels in children with lymphoproliferative disorders were significantly elevated as compared to controls. This was not observed in the case of malignant solid tumours. $\beta_2$-M elevated rates were 66.7% in patients with leukaemias and lymphomas and only 33.7% in solid tumours. The good response to antitumour therapy in the entire oncological group paralleled a significant decrease of pre-treatment $\beta_2$-M levels towards normal range ($p<0.001$).

Conclusions. $\beta_2$-M may serve as a useful diagnostic marker in paediatric lymphoproliferative disorders, but not in the case of malignant solid tumours. Monitoring of serum concentrations of $\beta_2$-M during oncological treatment may be of value, especially in children with lymphoproliferative neoplasms, however further studies are necessary in more homogenous and numerous group of patients.

Key words: $\beta_2$-Microglobulin, serum, diagnostics, treatment monitoring, malignant solid tumours, lymphoproliferative disorders, children

Poziomy $\beta_2$-miikroglobuliny w surowicy krwi w momencie rozpoznania i w trakcie terapii choroby nowotworowej u dzieci

Cel. Celem pracy była ocena klinicznej przydatności oznaczania surowiczego poziomu $\beta_2$-Miikroglobuliny ($\beta_2$-M) w diagnostyce i monitorowaniu efektów leczenia u dzieci z chorobą nowotworową.

Materiał i metody. Poziomy $\beta_2$-M w surowicy oznaczano przy użyciu metody ELISA w grupie 100 dzieci z rozpoznaniem ostrych białaczek i chloniaków złośliwych ($n=64$) oraz złośliwych guzów litych ($n=36$). Grupę kontrolną stanowiło 30 zdrowych dzieci. Poziom $\beta_2$-M oraz odsetki podwyższenych wartości $\beta_2$-M u pacjentów onkologicznych oznaczano prospektywnie na pięciu etapach choroby: przed leczeniem, w fazie częściowej i całkowitej klinicznej remisji choroby, po zakończeniu terapii oraz w okresie wznowy, bądź progresji nowotworu.

 Wyniki. Średni poziom $\beta_2$-M, oznaczony w momencie rozpoznania choroby u dzieci ze schorzeniami limfoproliferacyjnymi, znacząco przewyższał wartości stwierdzane u zdrowych dzieci, podczas gdy u pacjentów z rozpoznaniem nowotworów litych nie odbiegał od normy. Podwyższone wartości $\beta_2$-M stwierdzono u większości (66,7%) pacjentów z rozrostowymi schorzeniami układu krwiotwórczego i jedynie u 33,7% chorych z guzami litymi. W trakcie leczenia przeciwnowotworowego, wraz z osiąganą pozytywną odpowiedzią kliniczną, poziom $\beta_2$-M obniżył się do wartości prawidłowych ($p<0,001$).

Wnioski. Oznaczanie $\beta_2$-M może służyć jako wartościowy marker diagnostyczny u dzieci ze schorzeniami rozrostowymi układu krwiotwórczego, nie ma natomiast znaczenia w rozpoznawaniu złośliwych guzów litych wieku dziecięcego. Monitorowanie poziomów $\beta_2$-M w trakcie leczenia onkologicznego ma pewne znaczenie kliniczne, zwłaszcza u dzieci z chorobami limfoproliferacyjnymi, jednak spostrzeżenie to wymaga potwierdzenia w bardziej jednorodniej i liczniejszej grupie chorych.

Key words: $\beta_2$-Microglobulin, serum, diagnostics, treatment monitoring, malignant solid tumours, lymphoproliferative disorders, children

Słowa kluczowe: $\beta_2$-miikroglobulina, surowica, diagnostyka, monitorowanie terapii, złośliwe guzy life, schorzenia rozrostowe, dzieci

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**Introduction**

β₂-Microglobulin (β₂-M) is a low molecular weight non-glycosylated protein (11800 D) which constitutes the light chain of the class I major histocompatibility complex (MHC) [1]. It is present on the surface of most nucleated cells (mainly lymphocytes T, B and macrophages) [2] while membrane turnover is its principal source in blood and body fluids [3]. Immune activation results in an increased release of β₂-M into circulation and high levels of this protein have been found in a variety of infectious, inflammatory and autoimmune conditions [4-6]. The exact role of β₂-M in carcinogenesis and the reasons for its increased levels in the course of malignancy remain unknown. It has been shown that some tumours demonstrate decreased expression of β₂-M. This may serve as one of the mechanisms of escaping immune surveillance and progressing to metastases [7, 8]. However, significantly elevated serum β₂-M levels have been observed in numerous neoplasms, especially of the lymphoproliferative type, correlating with tumour mass, stage and progression. [9-12]. Clinical usefulness of β₂-M determination in most malignant solid tumours of adults was shown to be rather limited [13-15].

To the best of our knowledge, there have been no reports regarding the clinical significance of serum β₂-M in children with cancer. In view of the considerable histological, biological and clinical differences between childhood and adult malignancies, it seemed reasonable to investigate the issue. Thus, in the present study we have attempted to determine the significance of β₂-M measurements both in the diagnostics and in treatment monitoring of lymphoproliferative and malignant solid tumours in children.

**Material and method**

We enrolled 100 children with neoplastic disease, treated in the Division of Haematology and Oncology of the Department of Paediatrics, Haematology, Oncology and Endocrinology of the Medical University of Gdansk in Poland between the years 1995 and 2000. The group covered six histological types of childhood malignancies, i.e.: acute lymphoblastic leukaemia (ALL), acute non-lymphoblastic leukaemia (ANLL), non-Hodgkin lymphoma (NHL), Hodgkin’s disease (HD), Wilms’ tumour (Tu Wilms) and soft tissue sarcomas (SA). The diagnosis, staging, treatment and assessment of response to therapy were carried out in accordance to schemes provided by the International Society of Paediatric Oncology (SIOP) and Polish Paediatric Leukaemia/Lymphoma and Solid Tumours Study Groups for each particular type of malignancy. Pathological examinations were verified in two different institutions. Control group consisted of 30 healthy children. The clinical characteristics of patients and controls are shown in Table I.

In the case of patients with cancer the serum levels of β₂-M were determined in a prospective manner at five time points: before treatment (at diagnosis), in partial (PR) and complete clinical remission (CR), after therapy and during relapse or progression of cancer (PROG). The pre-treatment determination of β₂-M was carried out in both the entire oncological group and each type of neoplasm. Rates of elevated β₂-M measurements (>2.6 mg/L) were estimated for each type of malignancy and each phase of disease course. In the control group serum β₂-M levels were evaluated once after obtaining informed consent confirmed by parental signature.

The study had been approved by the Local Ethics Committee (decision nr 367/95).

**β₂-Microglobulin Assay**

Blood collected from patients and controls was centrifuged at 2000 rpm for 15 minutes to separate serum. Serum samples were stored frozen at −70°C until assayed. Measurements of β₂-M levels were performed in duplicate with the enzyme-linked immunosorbent assay (ELISA) (β₂-Microglobulin enzyme immunoassay, ref. nr 1131, Immunotech, France). The results were expressed in mg/L. To exclude a possible influence of renal impairment on serum β₂-M determinations, serum blood urea nitrogen (BUN) and creatinine level were checked. They were normal in all patients and controls at the time of sample collection.

**Statistical Analysis**

Statistical analysis was performed using Statistica 5.0 and S-PLUS software. Data distribution was checked with the Kolmogorow-Smirnow test, and all results of β₂-M determinations underwent statistical analysis with the use of non-parametric tests (Mann-Whitney U-test, Wilcoxon’s test). Statistical significance level was set for p<0.05.

**Results**

β₂-M levels and rates of its elevated values at diagnosis of malignant disease

Results of serum concentration of β₂-M at diagnosis of malignancy and in controls are reported in Table II.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Number (% of all)</th>
<th>Sex F/M</th>
<th>Mean age in years (age range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncological group</td>
<td>100 (100%)</td>
<td>45 / 55</td>
<td>6.9 (0.3-16.9)</td>
</tr>
<tr>
<td>Lympho-proliferative disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>38 (38%)</td>
<td>15 / 23</td>
<td>6.0 (2.0-14.8)</td>
</tr>
<tr>
<td>ANLL</td>
<td>3 (3%)</td>
<td>2 / 1</td>
<td>5.8 (3.4-10.0)</td>
</tr>
<tr>
<td>NHL</td>
<td>11 (11%)</td>
<td>1 / 10</td>
<td>9.1 (3.9-14.7)</td>
</tr>
<tr>
<td>HD</td>
<td>12 (12%)</td>
<td>5 / 7</td>
<td>12.4 (5.0-16.9)</td>
</tr>
<tr>
<td>Malignant solid tumours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tu Wilms</td>
<td>19 (19%)</td>
<td>11 / 8</td>
<td>4.1 (0.9-10.6)</td>
</tr>
<tr>
<td>SA</td>
<td>17 (17%)</td>
<td>11 / 6</td>
<td>7.0 (0.3-13.1)</td>
</tr>
<tr>
<td>Control group</td>
<td>30 (100%)</td>
<td>15 / 15</td>
<td>8.8 (2.3-16.6)</td>
</tr>
</tbody>
</table>
The values did not depend on sex or age. The median pre-treatment level of $\beta_2$-M determined for the entire malignancy group was significantly higher, as compared to healthy children (p<0.001). However, significant differences in pre-treatment $\beta_2$-M levels were observed, when analysed in particular histological types of neoplasms. We have found that the serum concentrations of $\beta_2$-M at the diagnosis of acute leukaemias and Hodgkin and non-Hodgkin lymphomas were significantly higher than in the control group (p<0.001 for ALL, p<0.05 for ANLL, NHL and HD). On the contrary, in children with Tu Wilms and SA the $\beta_2$-M levels did not differ from healthy controls (p>0.05). The rates of elevated initial $\beta_2$-M values for the entire group of cancer patients, lymphoproliferative disorders and solid neoplasms were of 55.7%, 66.7% and 33.7%, respectively.

$\beta_2$-M levels and rates of its elevated values at different stages of neoplastic disease

$\beta_2$-M serum concentrations in children with neoplasms at different stages of disease are shown in Table II. Median $\beta_2$-M concentration determined at diagnosis for the entire oncological group significantly exceeded the levels observed at complete clinical remission (CR) – both during treatment and after the termination of therapy (p<0.001 and p<0.05 respectively). It was shown that on obtaining CR of cancer, $\beta_2$-M concentrations decreased towards normal range. In the case of 14 patients with relapse or progression (PROG), the serum $\beta_2$-M levels returned to values similar to those found at diagnosis, however the difference between CR and PROG was not statistically significant (p>0.05).

Since we have shown significant differences in the pre-treatment $\beta_2$-M levels in children with lymphoproliferative disorders and malignant solid tumours, the changes in $\beta_2$-M levels during treatment in patients with ALL + ANLL + NHL + HD and with Tu Wilms + SA were analysed separately (Figures 1 and 2). The changes in $\beta_2$-M concentrations seem to reflect the activity and course of lymphoproliferative diseases, while their monitoring role in Tu Wilms and SA patients is uncertain.

Table II. The results of serum $\beta_2$-M at diagnosis and at different time points of oncological treatment as compared to healthy controls

<table>
<thead>
<tr>
<th>Stage of disease</th>
<th>(number of patients)</th>
<th>$\beta_2$-M (mg/l)</th>
<th>% of &gt; 2.6 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-treatment (n=61)</td>
<td>3.15 ± 1.70</td>
<td>2.80 (1.2 – 10.6)</td>
<td>55.7</td>
</tr>
<tr>
<td>ALL</td>
<td>3.67 ± 2.20</td>
<td>3.20 (1.2 – 10.6)</td>
<td>68.2</td>
</tr>
<tr>
<td>ANLL</td>
<td>2.80 ± 0.50</td>
<td>2.80 (2.3 – 3.3)</td>
<td>66.7</td>
</tr>
<tr>
<td>NHL</td>
<td>3.05 ± 0.90</td>
<td>3.20 (1.6 – 4.0)</td>
<td>66.7</td>
</tr>
<tr>
<td>HD</td>
<td>3.04 ± 0.98</td>
<td>2.15 (1.2 – 7.8)</td>
<td>30.0</td>
</tr>
<tr>
<td>Tu Wilms</td>
<td>2.78 ± 1.93</td>
<td>2.25 (1.2 – 4.6)</td>
<td>37.5</td>
</tr>
<tr>
<td>SA</td>
<td>2.53 ± 1.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>partial remission – PR (n=12)</td>
<td>3.51 ± 2.94</td>
<td>2.30</td>
<td>33.3</td>
</tr>
<tr>
<td>complete remission – CR (n=53)</td>
<td>2.23 ± 1.45</td>
<td>1.90</td>
<td>20.8</td>
</tr>
<tr>
<td>CR after treatment (n=58)</td>
<td>2.35 ± 0.91</td>
<td>2.25</td>
<td>29.3</td>
</tr>
<tr>
<td>progression – PROG (n=14)</td>
<td>3.51 ± 3.67</td>
<td>2.60</td>
<td>42.8</td>
</tr>
<tr>
<td>control group (n=30)</td>
<td>2.08 ± 0.49</td>
<td>2.05</td>
<td>6.7</td>
</tr>
</tbody>
</table>

** – p < 0.001 vs. control group
* – p < 0.05 vs. control group
# – p < 0.001 vs. CR
Ψ – p < 0.05 vs. after treatment

Figure 1. Changes in serum $\beta_2$-M levels during antitumour therapy in children with acute leukaemias and lymphomas

Figure 2. Individual courses of $\beta_2$-M levels in ten patients treated for Tu Wilms and SA
Discussion

The clinical applicability of serum β2-M concentration as a tumour marker in children with cancer has not yet been assessed. We attempted to find whether β2-M determination may be of value in the diagnostics and treatment monitoring in paediatric malignancies. Our data has shown that the pre-treatment serum concentration of β2-M, in the entire group of 100 cancer patients has significantly exceeded that found in healthy children. However, when studied separately in each histological type of neoplasm, the pre-treatment levels of β2-M behaved differently in lymphoproliferative and solid neoplasms. In children with ALL, ANLL, HD and NHL they were significantly higher than in controls, while in Tu Wilms and SA they remained within normal range. Similarly, the rates of elevated β2-M values in children with leukaemias and lymphomas and malignant solid tumours differed significantly (66.7% and 33.7%, respectively). These results are similar to those reported by other authors from studies performed on adult cancer patients. Initial serum concentrations of β2-M have been reported to be significantly increased in many types of adult lymphoproliferative disorders, such as: B-cell chronic lymphoblastic leukaemia, adult T-cell leukaemia, Hodgkin and non-Hodgkin lymphomas and multiple myeloma [9-12]. On the contrary, in adults with malignant solid tumours, the diagnostic usefulness of β2-M is rather limited [13-15]. It has been suggested, that such varied behaviour of β2-M concentration in haematological and solid malignancies may be caused by different biological sources of this protein. In leukaemias and lymphomas it may result from the relative overproduction of the β2-M chains or it may reflect the increased turnover of tumour cells [16]. It is postulated that in case of solid tumours increased β2-M levels reflect an enhancement of the immune system secondary to a malignant process [5, 16]. Perhaps these hypotheses might explain the significant differences between β2-M concentrations in lymphoproliferative and solid malignancies of childhood, stated in our study.

We attempted to find out whether the changes of β2-M concentrations in the course of antitumour therapy in paediatric patients correlated with the activity and clinical phase of cancer. The results observed in the entire oncological group, show that a good response to treatment is parallel to a significant decrease of the initial β2-M concentrations towards the normal range. Literature reports concerning the significance of β2-M in antitumour therapy monitoring are scarce and concerned exclusively with adult patients. Child et al. [10] have shown that chemotherapy in patients with NHL and HD causes a reduction in serum β2-M levels even before CR is achieved. The persistent elevation of β2-M reflects resistant disease, while its increasing concentrations reflect relapse. Our results are similar, although the relatively small number of patients forms a basic limitation of our study. There have been conflicting reports concerning β2-M monitoring in solid tumours in adults. Klein et al. [16] have proven that β2-M concentrations correlated with the clinical course of breast cancer in 365 women. On the contrary, Lotzniker et al. did not observe any significant differences in β2-M levels as measured in complete remission and progression among 186 patients with several types of cancer [15].

The latter data corresponds with the results of our study, in which, by monitoring β2-M levels we have failed to differentiate between CR and PROG phases of disease in childhood malignancies. It may not be ruled out that the reason for this phenomenon arises from the heterogeneity of the analysed group of patients, including both lymphoproliferative and solid neoplasms. It has been shown here, that the pre-treatment levels of β2-M-Microglobulin and the rates of its elevated values, behave in a different way in these two histological and clinical subgroups of neoplasms. The analysis of β2-M changes during the treatment of children with leukaemias, lymphomas and solid tumours has also shown that the individual patterns of β2-M levels differ in those groups of patients. Preliminary observations indicate that the significance of β2-M-Microglobulin in monitoring the treatment course of paediatric malignant solid tumours is rather limited, while it may be of particular value among children with acute leukaemias and lymphomas. However, to be able to make final conclusions, the monitoring of serum β2-M in numerous, homogenous group of children with neoplasm would be necessary.

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