NOWOTWORY Journal of Oncology • 2005 • volume 55
Number 1 • 34–38

Serum β_2-microglobulin levels at diagnosis and during antitumour treatment in children with malignant neoplasms

Ewa Bień[1], Anna Balcerska[1], Dominik Ciesielski[2]

Objective. *To assess whether serum β_2-Microglobulin (β_2-M) levels might serve as a marker for both diagnostics and treatment monitoring in children with cancer.*
Materials and methods. *Serum β_2-M levels and rates of β_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 β_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. β_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 parallelled a significant decrease of pre-treatment β_2-M levels towards normal range ($p<0.001$).*
Conclusions. *β_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 β_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.*

Poziomy β_2-mikroglobuliny w surowicy krwi w momencie rozpoznania i w trakcie terapii choroby nowotworowej u dzieci

Cel. *Celem pracy była ocena klinicznej przydatności oznaczania surowiczego poziomu β_2-Mikroglobuliny (β_2-M) w diagnostyce i monitorowaniu efektów leczenia u dzieci z chorobą nowotworową.*
Materiał i metody. *Poziomy β_2-M w surowicy oznaczano przy użyciu metody ELISA w grupie 100 dzieci z rozpoznaniem ostrych białaczek i chłoniaków złośliwych (n=64) oraz złośliwych guzów litych (n=36). Grupę kontrolną stanowiło 30 zdrowych dzieci. Poziom β_2-M oraz odsetki podwyższonych wartości β_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 β_2-M, oznaczony w momencie rozpoznania choroby u dzieci ze schorzeniami limfoproliferacyjnymi, znacząco przewyższał wartości stwierdzone u zdrowych dzieci, podczas gdy u pacjentów z rozpoznaniem nowotworów litych nie odbiegał od normy. Podwyższone wartości β_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 β_2-M obniżał się do wartości prawidłowych ($p<0,001$).*
Wnioski. *Oznaczanie β_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 β_2-M w trakcie leczenia onkologicznego ma pewne znaczenie kliniczne, zwłaszcza u dzieci z chorobami limfoproliferacyjnymi, jednak spostrzeżenie to wymaga potwierdzenia w bardziej jednorodnej i liczebnej grupie chorych.*

Key words: β_2-Microglobulin, serum, diagnostics, treatment monitoring, malignant solid tumours, lymphoproliferative disorders, children
Słowa kluczowe: β_2-mikroglobulina, surowica, diagnostyka, monitorowanie terapii, złośliwe guzy lite, schorzenia rozrostowe, dzieci

[1] Department of Paediatrics, Haematology, Oncology and Endocrinology
[2] Department of Immunopathology
Medical University of Gdansk, Poland

Introduction

β_2-Microglobulin (β_2-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 β_2-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 β_2-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 β_2-M. This may serve as one of the mechanisms of escaping immune surveillance and progressing to metastases [7, 8]. However, significantly elevated serum β_2-M levels have been observed in numerous neoplasms, especially of the lymphoproliferative type, correlating with tumour mass, stage and prognosis. [9-12]. Clinical usefulness of β_2-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 β_2-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 β_2-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 β_2-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 β_2-M was carried out in both the entire oncological group and each type of neoplasm. Rates of elevated β_2-M measurements (>2.6 mg/L) were estimated for each type of malignancy and each phase of disease course. In the control group serum β_2-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).

β_2-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 β_2-M levels were performed in duplicate with the enzyme-linked immunosorbent assay (ELISA) (β_2-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 β_2-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 Kołmogorow-Smirnow test, and all results of β_2-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

β_2-M levels and rates of its elevated values at diagnosis of malignant disease

Results of serum concentration of β_2-M at diagnosis of malignancy and in controls are reported in Table II.

Table I. Clinical characteristics of cancer patients and of the control group

Patients		Number (% of all)	Sex F / M	Mean age in years (age range)
Oncological group		100 (100%)	45 / 55	6.9 (0.3-16.9)
Lympho-proliferative disorders	ALL	38 (38%)	15 / 23	6.0 (2.0-14.8)
	ANLL	3 (3%)	2 / 1	5.8 (3.4-10.0)
	NHL	11 (11%)	1 / 10	9.1 (3.9-14.7)
	HD	12 (12%)	5 / 7	12.4 (5.0-16.9)
Malignant solid tumours	Tu Wilms	19 (19%)	11 / 8	4.1 (0.9-10.6)
	SA	17 (17%)	11 / 6	7.0 (0.3-13.1)
Control group		30 (100%)	15 / 15	8.8 (2.3-16.6)

Table II. The results of serum β_2-M at diagnosis and at different time points of oncological treatment as compared to healthy controls

Stage of disease (number of patients)	mean ± SD	β_2-M (mg/l) median (values range)	% of > 2.6 mg/l
pre-treatment (n=61)	3.15 ± 1.70	2.80 (1.2 – 10.6) $^{**\ \#\Psi}$	55.7
ALL	3.67 ± 2.20	3.20 (1.2 – 10.6)**	68.2
ANLL	2.80 ± 0.50	3.20 (1.2 – 10.6)*	66.7
NHL	3.05 ± 0.90	2.80 (2.3 – 3.3)*	66.7
HD	3.04 ± 0.98	3.20 (1.6 – 4.0)*	66.7
Tu Wilms	2.78 ± 1.93	2.15 (1.2 – 7.8)	30.0
SA	2.53 ± 1.23	2.25 (1.2 – 4.6)	37.5
partial remission – PR (n=12)	3.51 ± 2.94	2.30	33.3
complete remission – CR (n=53)	2.23 ± 1.45	1.90	20.8
CR after treatment (n=58)	2.35 ± 0.91	2.25	29.3
progression – PROG (n=14)	3.51 ± 3.67	2.60	42.8
control group (n=30)	2.08 ± 0.49	2.05	6.7

** – $p < 0.001$ vs. control group
* – $p < 0.05$ vs. control group
$^{\#}$ – $p < 0.001$ vs. CR
$^{\Psi}$ – $p < 0.05$ vs. after treatment

The values did not depend on sex or age. The median pre-treatment level of β_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 β_2-M levels were observed, when analysed in particular histological types of neoplasms. We have found that the serum concentrations of β_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 β_2-M levels did not differ from healthy controls ($p>0.05$). The rates of elevated initial β_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.

β_2-M levels and rates of its elevated values at different stages of neoplastic disease

β_2-M serum concentrations in children with neoplasms at different stages of disease are shown in Table II. Median β_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, β_2-M concentrations decreased towards normal range. In the case of 14 patients with relapse or progression (PROG), the serum β_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 β_2-M levels in children with lymphoproliferative disorders and malignant solid tumours, the changes in β_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 β_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.

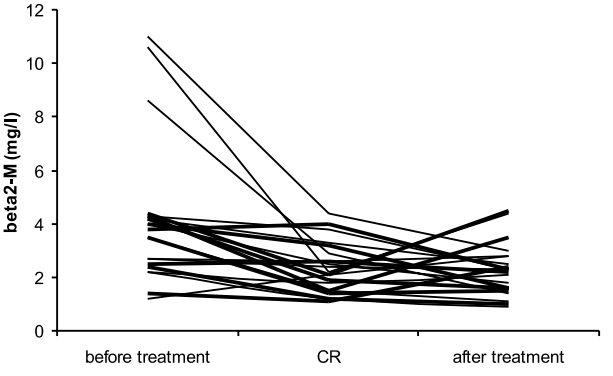

Figure 1. Changes in serum β_2-M levels during antitumour therapy in children with acute leukaemias and lymphomas

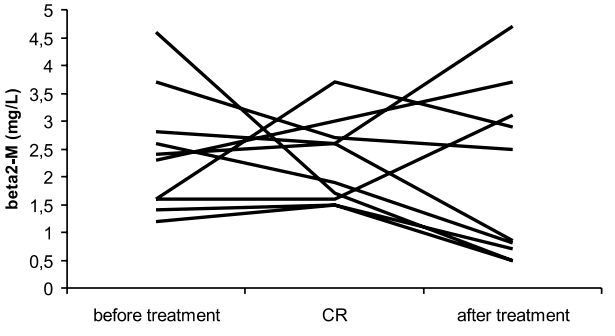

Figure 2. Individual courses of β_2-M levels in ten patients treated for Tu Wilms and SA

Discussion

The clinical applicability of serum β_2-M concentration as a tumour marker in children with cancer has not yet been assessed. We attempted to find whether β_2-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 β_2-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 β_2-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 β_2-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 β_2-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 β_2-M is rather limited [13-15]. It has been suggested, that such varied behaviour of β_2-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 β_2-M chains or it may reflect the increased turnover of tumour cells [16]. It is postulated that in case of solid tumours increased β_2-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 β_2-M concentrations in lymphoproliferative and solid malignancies of childhood, stated in our study.

We attempted to find out whether the changes of β_2-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 β_2-M concentrations towards the normal range. Literature reports concerning the significance of β_2-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 β_2-M levels even before CR is achieved. The persistent elevation of β_2-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 β_2-M monitoring in solid tumours in adults. Klein et al. [16] have proven that β_2-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 β_2-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 β_2-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 β_2-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 β_2-M changes during the treatment of children with leukaemias, lymphomas and solid tumours has also shown that the individual patterns of β_2-M levels differ in those groups of patients. Preliminary observations indicate that the significance of β_2-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 β_2-M in numerous, homogenous group of children with neoplasm would be necessary.

Ewa Bień MD, PhD
Department of Paediatrics, Haematology, Oncology and Endocrinology
Medical University of Gdańsk,
ul. Dębinki 7, 80-211 Gdańsk, Poland
e-mail: ebien@amg.gda.pl

References

1. Grey HM, Kubo RT, Colon SM et al. The small subunit of HLA antigens is β_2-microglobulin. *J Exp Med* 1973; 138: 1608-12.
2. Dorval G, Welsh KI, Nilsson K et al. Quantitation of β_2-microglobulin and HLA on the surface of human cells. I. T and B lymphocytes and lymphoblasts. *Scand J Immunol* 1977; 6: 255-63.
3. Karlsson FA, Groth T, Sege K et al. Turnover in humans of β_2-microglobulin: the constant chain of HLA-antigens. *Eur J Clin Invest* 1980; 10: 93-100.
4. Nilsson K, Evrin PE, Welsh KI. Production of β_2-microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplant Rev* 1974; 21: 53-84.
5. Kin K, Kasahara T, Itoh Y et al. β_2-Microglobulin production by highly purified human T and B lymphocytes in cell culture stimulated with various mitogens. *Immunology* 1979; 36: 47-54.
6. Bethea M, Forman DT. β_2-microglobulin: Its significance and clinical usefulness. *Ann Clin Lab Sci* 1990; 20: 163-8.
7. Doyle A, Martin WJ, Funa K et al. Markedly decreased expression of class I histocompatibility antigens, protein, and mRNA in human small-cell lung cancer. *J Exp Med* 1985; 161: 1135-51.
8. Garrido F, Gabrera T, Concha A et al. Natural history of HLA expression during tumour development. *Immunol Today* 1993; 10: 491-9.

9. Bataille R, Boccadoro M, Klein B et al. C-Reactive protein and β_2-microglobulin produce a simple and powerful myeloma staging system. *Blood* 1992; 80: 733-7.
10. Child JA, Spati B, Illingworth S et al. Beta2-microglobulin and C-reactive protein in the monitoring of lymphomas. *Cancer* 1980; 45: 318-26.
11. Molica S, Levato D, Cascavilla N et al. Clinico-prognostic implications of simultaneous increased serum levels of soluble CD23 and β_2-microglobulin in B-cell chronic lymphocytic leukemia. *Eur J Haematol* 1999; 62: 117-22.
12. Sadamori N, Mine M, Hakariya S et al. Clinical significance of β_2-microglobulin in serum of adult T cell leukemia. *Leukemia* 1995; 9: 594-7.
13. Engstr(m W. Serum levels and urinary excretion of beta-2-microglobulin in patients with urinary bladder carcinoma. *Eur Urol* 1988; 14: 218-21.
14. Johnson H Jr, Flye WM. Serum β_2-microglobulin determinations in patients bearing soft tissue sarcomas. *J Surg Oncol* 1983; 22: 175-8.
15. Lotzniker M, Pavesi F, Marbello L et al. Beta-2-microglobulin as a tumor marker in solid malignancies. *Oncology* 1988; 45: 162-5.
16. Klein B, Klein T, Figer A et al. Soluble histocompatibility antigen class I in breast cancer patients in relation to tumor burden. *Cancer* 1991; 67: 2295-99.

Paper received: 5 April 2004
Accepted: 14 June 2004