

BETA-2 MICROGLOBULINE BIOLOGICAL MARKER WITH DIAGNOSTIC AND PROGNOSIS VALUE IN MULTIPLE MYELOMA

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REZUMAT

După o scurtă prezentare a beta-2 microglobulinei (β 2m) sub raport biochimic, funcțional și aplicativ, printr-un studiu de tip prospectiv, autorii își propun să urmărească corelația existentă între valorile serice ale β 2m și principalii indicatori biologici cu valoare diagnostică și prognostică în grupul nosocomial al gamapatiilor monoclonale. Informațiile adunate de la un număr de 41 de bolnavi au fost prelucrate statistic cu ajutorul coeficientului de corelație "r" și a testului "chi" pătrat. Analiza supraviețuirii s-a efectuat folosind curbele Kaplan-Meier și regresia Cox, utilizând programul SPSS 10. Datele obținute au permis stabilirea unei corelații semnificative de tip direct între β 2m și următorii parametri biologici, reprezentativi pentru evaluarea volumului masei celulare tumorale: procentul plasmocitelor medulare, valoarea proteinelor serice, rata de sinteză a Ig mielomatoase, nivelul calciului seric, a creatininei serice, a VSH-ului. O corelație semnificativă de tip invers a rezultat din relația β 2m și valoarea hemoglobinei. O valoare prognostică din punct de vedere al supraviețuirii au avut-o creatinina serică, hemoglobina și mai ales beta-2 microglobulina.

Cuvinte cheie: β 2m, mielom multiplu, masă tumorală, coeficient de corelație

ABSTRACT

After a short presentation of beta-2 microglobuline (β -2m) from a biochemical and functional point of view, the authors aim to find a correlation between serum level of β 2m and the main biological markers with diagnostic and prognosis value in monoclonal gammopathies. The evidences gathered from 41 patients were studied with the coefficient of correlation "r" and with the "chi" square test. Survival analyses were performed using Kaplan-Meier curves and Cox regression. These analyses were done with SPSS 10 statistical software. The results obtained allowed to establish a direct significant correlation between the following parameters evaluating the tumour mass: the percent of bone marrow plasma cells, serum proteins, and the synthesis rate of monoclonal immunoglobulins, serum calcium, serum creatinine and ESR. A reverse significant correlation resulted from the association between β 2m and serum hemoglobin. A prognostic significance from the survival point of view has had serum creatinine, haemoglobin and especially beta-2 microglobuline.

Key Words: β -2microglobulin, multiple myeloma, tumour mass, correlation coefficient

INTRODUCTION

In the 37 years that have passed since first being isolated (1968) β -2m has got a well defined place in both biochemical and clinical applications.

β -2m along with the heavy chain α is a HLA class I molecule structure. First considered as a leucocyte alloantigen, HLA class I molecules have proved to have determinative role in allografts rejection, therefore

the name of histocompatibility antigen. β -2m or the light chain β is situated extracellularly and is linked by noncovalent bonds with the α_3 domain of the α heavy chain; it has in its structure a single domain of 99 aminoacids, is invariant in conformation and shows no polymorfism. Its high affinity for the α_1 and α_2 domains of the α heavy chain makes the HLA class I molecule stable. β -2m has a molecular weight of 11800 and is encoded by a gene on chromosome 15.

The human leucocyte antigens are encoded by the major histocompatibility complex (MHC), a cluster of genes located on the short arm of chromosome 6. The first two classes of genes (HLA-A, B,C and HLA-D, HLA-DR, HLA-DQ, HLA-DP) survey the tissue histocompatibility and the third class participates in the immune response, but not in histocompatibility, encoding the complement components C_2 , C_4A , C_4B , Bf (properdine), 21-hidroxilase A and B and tumor necrosis factor α and β .

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HLA class I molecules are present not only on leucocytes surface but on platelets and on all nucleated cells. HLA class 2 molecules are more restricted in their tissue distribution than class I antigens, and are primarily found on B lymphocytes, macrophages, monocytes, endothelial cells, activated T lymphocytes and Langerhans cells.¹⁻³

From a clinical point of view there are two situations in which the serum or urinary levels of β -2m are used as an evaluation of the tumour mass, or of the renal function:

- The ubiquity of HLA class I molecules on cells membranes makes the levels of β -2m in sera and other fluids to be in correlation with the mass of proliferating cells;^{4,5}

- In processes that affect the glomerulus (glomerulonephritis, diabetic nephropathy) the serum level of β -2m will be significantly higher because 95% of β -2m passes the glomerular filter, whereas when the proximal tubule is injured the excretion of β -2m is higher, 99% of the β -2m filtered being reabsorbed at this level.⁶⁻¹⁰

The aim of this study is to evaluate the degree of correlation between serum β -2m and the markers accepted as significant in tumour mass evaluation and the prognostic significance of β -2m in multiple myeloma.

MATERIAL AND METHODS

The authors evaluated through a prospective longitudinal study the correlation between β -2m and the main biological markers with prognostic and diagnostic value for the disease characterised by the presence of the monoclonal M component and the neoplastic proliferation of plasma cells. The study involved 41 patients with multiple myeloma diagnosed between January 2001 and June 2005. The median age of patients in the study group was 66 years (range, 25 to 85 years). There were 18 males and 23 females. Considering the type of multiple myeloma, 59% were IgG, 33% IgA, 2% IgM and 6% were micromolecular. Clinical stage according to Durie and Salmon was distributed as follows: 3% stage I, 19% stage II, and 78% stage III.

Serum samples were drawn at diagnosis in 37% of cases, in the plateau phase in 56%, and 7% of patients had progressive disease. Patients were treated with melphalan and prednisone, except those with progressive disease who followed VAD regimens. In order to measure β -2m levels an IMMULITE analyser was used; the anti β -2m antibodies were delivered in

vials of 100 test units (LKBM) by EURO/DPC- Los Angeles. The upper reference limit of the healthy controls was 1.73 mg/dl. The statistical evaluation of the data was performed on computer; the purpose being the evaluation of between two variables, the "r" correlation coefficient (Bravais-Pearson) and the χ^2 test were used.⁷ Survival analyses were performed using Kaplan-Meier curves and Cox regression. These analyses were done with SPSS 10 statistical software. A p value less than 0.05 was considered statistically significant.

RESULTS

48.7% of patients had higher than normal β -2m levels. The abnormal values were found in patients in III stage (87%) and II stage (13%) of the disease. 88% of these patients were over 50 years of age.

Between the serum β -2m higher than, 1.73 mg/l and the bone marrow plasma cells there is a correlation coefficient of +0.45 (direct medium correlation), whereas at values lower than 1.73 mg/dl the correlation coefficient was +0.13 (direct, insignificant correlation).

When levels of β -2m >1.73 mg/l were compared with serum protein levels the correlation coefficient was +0.41 (direct, medium correlation) whereas at levels < 1.73 mg/l the correlation was negligible ($r = 0.04$).

Comparing levels of β -2m >1.73 mg/l with the parameters above mentioned (% of marrow plasma cells and serum proteins) we obtained a strong and direct correlation ($r = +0.70$).

Levels of β -2m > 1.73 mg/l compared with the association between percentage of marrow plasma cells and ESR showed a strong and direct correlation ($r = 0.77$).

Comparing values of β -2m > 1.73 mg/l with the levels of myeloma immunoglobulin we observed a medium direct correlation ($r = 0.38$) and an insignificant correlation ($r = 0.02$) when we looked at values lower than 1.73 mg/l.

Levels of β -2m >1.73 mg/l compared with serum creatinine showed a direct strong correlation ($r = 0.87$), but the correlation was negligible at levels < 1.73 mg/l ($r = +0.18$).

β -2m > 1.73 mg/l, compared with ESR, demonstrated a medium correlation ($r = +0.42$), but the correlation was insignificant at a β -2m <1.73 mg/l.

Levels of β -2m > 1.73 mg/l correlated medium and inverse with the hemoglobin levels at levels < 1.73 mg/l the correlation was weak and inverse.

Levels of β -2m > 1.73 mg/l, compared with the

serum calcium, showed a direct weak correlation ($r = +0.22$) the same as at levels $< 1,73$ mg/l.

The medium survival time of the patients was 52 months. Among the parameters studied serum creatinine, hemoglobin and β -2m were found to have a statistically significant prognostic value.

Patients with creatinine lesser than 2 mg/dl had a median survival of 60 months, and those with creatinine greater than 2 mg/dl had a median survival of 11 months.

Patients with severe anemia (Hb < 8 g/dl) had a median survival of 41 months, and those with mild to moderate anemia survived longer (median of 91 months).

Regardless of the clinical stages patients with high levels of β -2m had a poorer survival. A statistically significant cut off level was established at 4 mg/dl. Those with β -2m lesser than 4 mg/dl had a medium survival of 65 months, and those with β -2m greater than 4 mg/dl had a medium survival of 9 months ($p = 0.018$). Even when considering only patients in stage III, β -2m still has a statistically significant prognostic value. Those with β -2m < 4 mg/dl survived longer (median of 24 months) than the others (median of 9 months) ($p = 0.022$).

DISCUSSION

If the presence of β -2m on the surface of all nucleated cells, as a component of the HLA class I molecule, can be associated with the notion of histocompatibility and thus with the immune response, the presence of β -2m on the surface of the numerous tumour cell lines (MM, MW, CLL, NHL) identifies it with the notion of tumour mass.

The relationship β -2m - tumour mass, in the case of multiple myeloma (MM), can be restricted to the evaluation of two main parameters: the degree of bone marrow plasmocytosis and the synthesis rate of myeloma immunoglobulins, and of the subsequent biologic markers: ESR, serum calcium, serum creatinine, the extension of bone lesions.⁸⁻¹²

In our patients the mentioned relationship is confirmed by the direct or inverse correlations demonstrated. Thus the higher levels of β -2m showed a direct medium or strong correlation with marrow plasma cells, serum protein, monoclonal immunoglobulin, serum calcium, serum creatinine and ESR, and an inverse correlation with the hemoglobine levels.

The strong correlation between β -2m and serum creatinine could not be associated with a glomerular

lesion or with a significant proteinuria to justify the raise of serum creatinine due to the lower glomerular filtration. Likewise the ESR values remained without further evaluation. If the involvement of interleukine 6 in the raise of ESR could not be evaluated, the participation of C reactive protein could be estimated. The relationship ESR/ IL 6/ CRP could be seen through the key position that IL 6 has in the proliferation of the myeloma cells, in the osteoclasts activation and in the synthesis of the acute phase proteins by the hepatocytes. β -2m proved to be a very important prognostic value, in this case better than the Durie-Salmon staging system. The study group being small further investigations are needed to confirm these results. The lack of financial support does not allow the regular determination of β -2m levels and a better synchronization of the specific investigations that are able to offer an instant of the clinical evolution, which in a prospective longitudinal investigation, allows the evaluation of the relationship between various biological markers and the course of the disease.

CONCLUSIONS

β -2m significantly correlates with tumour mass both at the time of diagnosis and at the end of therapeutic protocol.

β -2m together with the clinical criteria for the disease staging enable a better prognostic orientation; lower serum levels correlates with a better prognosis, with a longer remission and survival. Correlating β -2m levels with CRP, LDH, and serum albumin assures a better prognosis assessment.

Thus, from a minimal panel of biological investigations beside β -2m, other information is needed such as that offered by the serum proteins electrophoresis (sometimes even urinary proteins electrophoresis- the case of Ig D MM or micromolecular MM), immunoelectrophoresis and quantitative determination of immunoglobulins. As in numerous other studies β -2m proved to be a good prognostic factor in multiple myeloma.¹¹⁻¹⁷

The lack of the synchronization of the investigations, lack of periodical evaluation, most of the time due to financial reasons, affects the therapeutic decisions based on objective evidence.

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