THE CORRELATION OF SERUM IMMUNOGLOBULIN FREE LIGHT CHAIN LEVELS AND SELECTED BIOLOGICAL MARKERS IN MULTIPLE MYELOMA

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Aims: the presented study is aimed at the evaluation of correlation of free light chains serum levels – kappa, lambda and their relation (K/L ratio) and serum levels of selected biological markers in a group of patients with multiple myeloma examined at the time of the diagnosis.

Methods: 102 patients with multiple myeloma were included in this prospective study. Free light chains serum levels were determined by Freelite™ Binding Site system, for determination of serum levels of selected parameters the following methods were used: REA thymidinekinase (TK), RIA (β_2 microglobulin (β_2m), ICTP, PINP), enzymeimmunoassay (sIL-6R, sVCAM-1, sICAM-1, sOPG) and quantitative enzymatic immunoassay (sHGF, sVEGF, sSyn-1) a sFas).

Results: There was found a correlation in the kappa-group of the dominant kappa chain and serum readings of β_2m ($r = 0.344$, $p = 0.005$), TK ($r = 0.263$, $p = 0.035$), ICTP ($r = 0.402$, $p = 0.001$), PINP ($r = 0.264$, $p = 0.039$), sOPG ($r = 0.328$, $p = 0.028$), sSyn-1 ($r = 0.255$, $p = 0.046$) and sFas ($r = 0.418$, $p = 0.001$). In case of K/L ratio there was found a statistically significant association of levels of β_2m ($r = 0.316$, $p = 0.01$), TK ($r = 0.274$, $p = 0.027$), ICTP ($r = 0.346$, $p = 0.006$), PINP ($r = 0.261$, $p = 0.042$), sSyn-1 ($r = 0.283$, $p = 0.026$) and sFas ($r = 0.377$, $p = 0.002$). In the lambda-group the analysis confirmed mutual dependence of the dominant lambda chain levels on β_2m ($r = 0.476$, $p = 0.003$), ICTP ($r = 0.375$, $p = 0.022$), sVCAM-1 ($r = 0.383$, $p = 0.019$), sHGF ($r = 0.441$, $p = 0.006$) and sFas ($r = 0.334$, $p = 0.040$). In addition we ascertained a correlation of L/K ratio and levels of β_2m ($r = -0.473$, $p = 0.003$), TK ($r = -0.412$, $p = 0.011$), ICTP ($r = -0.331$, $p = 0.045$), PINP ($r = -0.409$, $p = 0.012$), sHGF ($r = -0.357$, $p = 0.028$), sSyn-1 ($r = -0.449$, $p = 0.005$) a sFas ($r = -0.371$, $p = 0.022$).

Conclusions: The study confirmed mutual correlation of FLC serum levels and the levels of several selected biological markers, in particular β_2m , TK, ICTP, PINP, sSyn-1 a sFas at time of the diagnosis. It referred to the mutual relation of bone marrow microenvironment, biological qualities of clonal plasmocytes and the intensity of the free light chains production by the tumour cell population.

INTRODUCTION

Multiple myeloma (MM) is a malignant hematological disease characterised by the uncontrolled proliferation and accumulation of neoplastically transformed plasma cells, production of monoclonal immunoglobulin (MIG) detectable in the serum and/or urine and various degrees of organ involvement (hypercalcemia, renal insufficiency, osteolytic bone lesions or osteoporosis, anemia and immunodeficiency)^{1, 2}. Apart from the complete molecule of monoclonal immunoglobulin, the plasma cells also produce single free light chains (FLC) which are not fixed to the MIG molecule³. In the last few years, the assessment of free light chain serum levels has become an irreplaceable part of the examination algorithm in diagnostics, screening and evaluation of treatment response not only in patients with multiple myeloma but also in other malignant or potentially malignant monoclonal

gammopathies^{4, 6}. The bone marrow microenvironment in relation to the pathological plasmacellular infiltration is characterised by a very large system of intercellular and cytokine interactions among the stromal cells of bone marrow, bone elements and tumour cells themselves, in close relation to their own biological qualities of the disease⁷. Some unusual biochemical parameters appear to be very promising as possible future markers not only in the neoplastic transformation of potential malignant monoclonal gammopathy of undetermined significance (MGUS) and its differentiation from the early stage MM but also as markers of advanced stage, progression and monitoring of treatment response in patients with multiple myeloma⁸⁻¹⁰. The aim of this study was to determine the relation of FLC serum levels to 12 selected less usual biological parameters in patients with multiple myeloma who were examined at the time of diagnosis.

MATERIALS AND METHODS

The sample studied included 102 patients examined at the time of diagnosis and who fulfilled the SWOG and IMWG criteria for multiple myeloma diagnosis¹¹. The age range was 43-82, median 63 years, men and women ratio 1:1.04. Immunochemical type IgG was found in 58 % (n = 60), IgA 22 % (n = 22), Bence - Jones 17 % (n = 17), IgD 1 % (n = 1) and nonsecretoric type 2 % (n = 2). The kappa light chain secretion was found in 68 % (n=69) and lambda secretion in 32 % (n = 33) of patients. With the use of Durie-Salmon staging system there 12 % (n = 12) of patients were in stage I, 28 % (n = 29) in stage II and 60 % (n = 61) in stage III. The A substage (creatinine under 177 $\mu\text{mol/l}$) was found in 75 % of patients¹². Using the International Prognostic Index (IPI) staging system 12 % (n = 12) of patients were in the 1st stage, 22 % (n = 22) in the 2nd stage and 66 % (n = 68) in the 3rd stage¹³. Serum levels of free light chains were set by the FreeliteTM Binding Site system (Immunotech, standard serum interval: kappa 3.3-19.4 mg/l, lambda 5.7-26.3 mg/l, lambda /kappa (K/L ratio) 0.26-1.65) (ref.³). Serum levels were determined by the following methods: β_2 microglobulin ($\beta_2\text{m}$) by the radioimmunoanalysis method (RIA, standard range 0-2.5 mg/l), thymidine kinase (TK) by radioenzymatic method (REA, 0-6 IU/l), intercellular C-terminal telopeptide collagen-I (ICTP, m: 2.1-5.0, w: 2.1-5.6 $\mu\text{g/l}$) and N-terminal peptide procollagen-I (PINP, m: 21-78, w: 19-102 $\mu\text{g/l}$) by RIA method; vascular cell adhesive molecule-1 (sVCAM, 395-714 ng/ml), intercellular cell adhesive molecule-1 (sICAM, 269-691 ng/ml), soluble form of IL-6R (sIL-6R, 10-90 ng/ml) and soluble osteoprotegerine (sOPG, 3.7-4.4 $\mu\text{mol/l}$) by enzymeimmunoassay method; hepatocyte growth factor (sHGF, 671-1992 pg/ml), vascular endothelial growth factor (sVEGF, 62-707 pg/ml), syndecan-1/sCD138 (sSyn-1, 37-123 ng/ml) and Fas antigen (sFas 4792-1750 pg/ml) were determined by a quantitative sandwich enzymatic immunoassay method. For statistical evaluation the Spearman Rank Correlation Coefficient test was used. Kappa and lambda secretion, respectively kappa and lambda groups were assessed separately due to the reciprocal relation of serum levels and K/L ratio of free chains.

RESULTS

Abnormal serum levels of free light chains were recorded in 92 % of patients and, a pathological K/L ratio was found in 91 % cases. Abnormal $\beta_2\text{m}$ levels were recorded in 95.1 %, in TK in 58.8 %, sIL-6R in 68.6 %, sVCAM in 74 %, sICAM in 32.6 %, ICTP in 72.7 %, PINP in 21 %; abnormal levels (under and above standard interval) were recorded in case of sOPG in 97.3 %, sHGF in 39.3 %, sVEGF in 7.7 %, sSyn-1 in 69.4 % and in case of sFas antigen in 3.8 %. A significant correlation of serum levels of the kappa dominant chain and serum levels of $\beta_2\text{m}$ ($r = 0.344$, $p = 0.005$), TK ($r = 0.263$, $p = 0.035$), ICTP ($r = 0.402$, $p = 0.001$), PINP ($r = 0.264$, $p = 0.039$),

sOPG ($r = 0.328$, $p = 0.028$), sSyn-1 ($r = 0.255$, $p = 0.046$) and sFas ($r = 0.418$, $p = 0.001$) were found. In the case of the K/L ratio and particular parameter assessment, a correlation of the levels of $\beta_2\text{m}$ ($r = 0.316$, $p = 0.01$), TK ($r = 0.274$, $p = 0.027$), ICTP ($r = 0.346$, $p = 0.006$), PINP ($r = 0.261$, $p = 0.042$), sSyn-1 ($r = 0.283$, $p = 0.026$) and sFas ($r = 0.377$, $p = 0.002$). In the lambda group the correlation analysis confirmed mutual dependence of the dominant lambda chain level and levels of $\beta_2\text{m}$ ($r = 0.476$, $p = 0.003$), ICTP ($r = 0.375$, $p = 0.022$), sVCAM-1 ($r = 0.383$, $p = 0.019$), sHGF ($r = 0.441$, $p = 0.006$) and sFas ($r = 0.334$, $p = 0.040$). Likewise, an association of K/L ratio and the levels of $\beta_2\text{m}$ ($r = -0.473$, $p = 0.003$), TK ($r = -0.412$, $p = 0.011$), ICTP ($r = -0.331$, $p = 0.045$), PINP ($r = -0.409$, $p = 0.012$), sHGF ($r = -0.357$, $p = 0.028$), sSyn-1 ($r = -0.449$, $p = 0.005$) and sFas ($r = -0.371$, $p = 0.022$) was revealed. No significant correlation of alternative chain levels and monitored parameters levels was found for any of the groups.

DISCUSSION

The results show the mutual relation of serum levels of FLC, K/L ratio and selected untraditional markers of multiple myeloma. The abnormal percentage of serum free light chain levels and K/L ratio for the analysed group approximately corresponds to previously published studies which have reported comparable pathological percentages of FLC even if there was a higher percentage in the case of the K/L ratio than FLC levels themselves in most studies^{4, 14, 15}. The correlation of FLC serum levels, K/L ratio and $\beta_2\text{m}$ levels not only results from the biological qualities of both parameters (level increase in case of renal insufficiency, polymerization), but also both parameters are markers of the size of the tumour mass. The interval levels of $\beta_2\text{m}$, the most important prognostic factor in multiple myeloma, are the new staging systems components - International Prognostic Index (IPI), South-West Oncology Group (SWOG) (ref.^{12, 16}); there was a correlation of these system stages and FLC levels, also the combination of IPI system and serum levels of FLC and K/L ratio appears to be very promising prognostic marker¹⁷⁻²⁰. The relation of FLC and serum thymidinekinase as proliferation markers and as tumour mass markers is explainable as well; an association was found of FLC serum levels and OLOMOUC staging system phases in which $\beta_2\text{m}$ and TK serum levels were used²¹⁻²³. The detected relation of the bone metabolism parameters ICTP, PINP and FLC levels can also be explained by the association of these markers to the stage of bone involvement and also to the progress and prognosis of the disease^{24, 25}. Detected relation of FLC, K/L ratio serum levels and Syndecan-1 confirms the unique position of this less usual parameter. Syndecan-1 is a proteoglycan released from the surface of viable malignant plasma cells, mainly concerned with the proliferation process, apoptosis of tumour and also bone cells and relating bone involvement; influences the cytokine net in direct and indirect way in conditions of

the bone marrow microenvironment. It is considered to be one of the most important molecules in the multiple myeloma pathobiology²⁶. Syndecan-1 appears to be a new important prognostic marker, also a possible parameter for discernment of monoclonal gammopathy of undetermined significance and multiple myeloma^{8, 9, 27-29}.

Detected correlation of FLC, K/L ratio and sFas levels is now more of the academical meaning which would be observed. Soluble Fas antigen is a part of antiapoptotic mechanisms participating in the immortality of tumour cell line³⁰.

CONCLUSIONS

The results confirm the relation of serum levels of free light chain kappa, resp. lambda and K/L ratio and several less usual parameters in multiple myeloma. The study confirmed the association with tumour mass, biolocal qualities of myeloma cells, parameters of bone metabolism, myeloma bone disease and the bone marrow microenvironment itself. The conclusions of the study give source materials for use of less usual markers for distinguishing monoclonal gammopathy of undetermined significance from multiple myeloma and for evaluation of progress and treatment response in patients with MM. Kappa/Lambda ratio particularly seems to be more sensitive marker than free light chains levels.

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