SERUM FREE LIGHT CHAIN RATIO AND ITS CORRELATION WITH MARKERS OF TUMOR BURDEN AND PROGNOSIS AT INITIAL DIAGNOSIS OF MULTIPLE MYELOMA

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Abstract: Multiple myeloma is the most frequent malignant monoclonal gammopathy characterized by plasma cell proliferation and monoclonal M-protein production as consequence. Gold standard for diagnosis of monoclonal gammopathies are urinary and serum electrophoretic tehniques. Until recently, multiple myeloma was defined and diagnosed by damaged organs, particularly through hypercalcemia, renal failure, anemia and bone lesions. International Myeloma Working Group revised the criteria for multiple myeloma diagnosis by adding three biomarkers of malignancy effective in early recognition of patients who have not developed permanent organ damage. Those biomarkers are clonal proliferation of plasmocytes in bone marrow ≥60%, ratio of serum free light chains ≥100 with the included serum free light chain having an absolute concentration >100 mg/L and more than one osseus change on magnetic resonance imaging. This enables early beginning of an effective therapy in high-risk patients. Objective of the research was to evaluate the impact of serum free light chain ratio, total proteins and serum protein electrophoresis, as an initial panel for diagnosis of multiple myeloma, on beta 2 microglobulin and albumin, as markers of tumor burden and prognosis, at baseline and the differences between analyzed parameters in study and control group. Design of the research was a cross sectional study/study of prevalence conducted in the period of two months. Study group consisted of 7 newly diagnosed patients, before treatment, at 18 to 80 years of age. Control group consisted of 10 healthy individuals. All participants were included in the research after prior written informed consent. The concentration of serum free light chains (N-Latex Free Light Chains) and beta 2 microglobulin were determined nephelometrically on the plasma protein analyzer BN proSpec-Siemens (Siemens Medical Systems). The ratio was calculated by the analyzer software. Any result of the ratio <0.26 and >1.65 was considered pathological, depending on the involved component. Significance was determined at p<0.05. Electrophoretic examination of serum proteins was performed by gradient (4-22%) sodium dodecyl sulfate polyacrylamide gel electrophoresis. The confirmation of the diagnosis was obtained from the hospital information system of the Hematology Clinic in Skopje. For R = 0,99 and p<0,01 we found a high correlation between beta 2 microglobulin and serum free light chain ratio, total proteins and serum protein electrophoresis at baseline. For R = 0,98 and p<0,05 there was a high correlation between albumin and serum free light chain ratio, total proteins and protein electrophoresis in serum at baseline. Serum free light chain ratio results were significantly higher in study group p<0,05 (p=0,01). Beta 2 microglobulin results were significantly higher in study group p<0,01 (p=0,001). In conclusion, the results of the study confirmed the association between serum free light chain ratio and beta 2 microglobulin as an indicator of tumor burden in multiple myeloma, as well as with the biological characteristics of myeloma cells.
Keywords: serum free light chain ratio

1. INTRODUCTION

The main feature of multiple myeloma-MM is plasmacytosis and production of abnormal monoclonal immunoglobulin (Kyle, 1999). Malignant plasmocytes and their monoclonal immunoglobulin products so-called M-protein or paraprotein, cause tissue and organ damage, typical of the clinical picture of MM such as bone manifestations, renal involvement, anemia and hypercalcemia (International Myeloma Working Group, 2003). There is no single test for diagnosis and monitoring of plasma cell dyscrasias (Katzmann et al, 2009). Routine practice in the diagnosis of monoclonal gammopathies is determination of the urinary or serum paraprotein, by electrophoretic procedures such as electrophoresis or immunofixation electrophoresis (Willrich & Katzmann, 2016), but the sensitivity is limited in cases of low concentration of the monoclonal component or only the presence of free light chains (Oliveros Conejero, Pascual Usandizaga & Garrido Chércoles, 2020). Nephelometric methods can help detect M-protein by compensating for some of the shortcomings of electrophoretic techniques. In the past, MM was recognized and diagnosed by damaged organs, particularly through hypercalcemia, renal failure, anemia and bone

lesions (CRAB features) resulting from the clonal proliferation. In 2014, International Myeloma Working Group revised the criteria for MM diagnosis by adding three biomarkers of malignancy effective in early recognition of patients who have not developed CRAB features (Rajkumar et al, 2014). Those biomarkers are clonal proliferation of plasmocytes in bone marrow ≥60%, serum free light chain ratio-sFLC ratio ≥100 where included serum free light chain-sFLC have an absolute concentration >100 mg/L and more than one osseus change on magnetic resonance imaging. This enables early beginning of treatment and prevention of permanent organ impairment in high-risk patients. The specific indicators are independently related with progression to symptomatic organ damage of approximately 80% demonstrated in two or more different studies (Rajkumar et al, 2014).

From the previous research in this area, the largest number of studies and the insights from them relate to the utility of sFLC in the recognition of MM and monitoring the effect of therapy, especially in oligo-secretory and non-secretory MM (Mead et al, 2004) and related monoclonal gammopathies. Several studies have shown that sFLC determination has a significant correlation with tumor burden compared to Bence Jones proteinuria (Bradwell, Carr-Smith, Mead, Harvey, & Drayson, 2003).

The objective of the research was to evaluate the impact of sFLC-ratio, serum total proteins-TP and serum protein electrophoresis-SPEP on beta 2 microglobulin-B2M and serum albumin-ALB at the initial diagnosis of MM and to determine the difference in the analyzed parameters between the studied and control groups.

2. MATERIAL AND METHODS

Design of the research was a study of prevalence conducted in the period from 15.08.2022 to 15.10.2022. Inclusion criteria: all MM patients at initial diagnosis, 18 to 80 years of age, male and female who were not on a therapy protocol. Exclusion criteria: all inflammatory conditions besides MM, menstrual bleeding, pregnancy. The study group consisted of 7 (N=7) newly diagnosed patients with MM. Control group were 10 (N=10) healthy individuals. Participants were included in the research after prior written informed consent. Newly diagnosed MM patients and healthy controls were analyzed by the monoclonal protein test panel (TP, SPEP and sFLCratio), and tumor burden was assessed by determination of the concentration of B2M and ALB. SPEP was performed by gradient (4-22%) sodium dodecyl sulfate polyacrylamide gel electrophoresis SDS-PAGE. TP were determined photometrically (Biuret method). ALB were determined photometrically (Bromcresol Green-BCG-method). TP and ALB in serum were measured on the Cobas c501 biochemical analyzer (Roche Diagnostics). The concentration of sFLC (N-Latex FLC) and B2M were determined nephelometrically on a plasma protein analyzer BN proSpec-Siemens (Siemens Medical Systems). sFLCratio was calculated mathematically by the analyzer software itself. Any sFLCratio result <0.26 and >1.65, depending on the involved component, was considered pathological. For the purposes of the research, venous blood was used, which was collected in a test tube without anticoagulant (6 ml) or with gel to obtain serum. The confirmation of the diagnosis of MM was obtained from the hospital information system of the Hematology Clinic in Skopje. SPEP was performed at the Institute of Medical and Experimental Biochemistry at the Faculty of Medicine in Skopje. sFLC, FLC ratio, B2M, ALB, TP were performed at the University Institute of Clinical Biochemistry-Skopje.

Statistics: For data processing we used STATISTICA 7.1. Measure of significance was $p<0.05$. The data are shown in tabular form.

3. RESULTS

Correlation

sFLC-ratio, TP, SPEP and B2M

Between B2M as a dependent variable and sFLC-ratio, TP, SPEP, as independent variables for R = 0.99 there was determined a very strong significant ($p<0.01$) correlation (Table 1). The greatest influence on B2M had SPEP Beta = - 1.84, then sFLC-ratio Beta = - 1.26, and the weakest was the influence of TP (serum) Beta = 0.21. Patients with pathological finding on SPEP had on average 50.60 mg/L lower value of B2M compared to patients with normal finding, significant for p=0.004, with unchanged values of other parameters.

With each unit value increase in sFLC-ratio, B2M (on average) decreased by 0.11 mg/L, significant at p=0.004, at unchanged values of other parameters.

With each unit value increase in TP (1 g/L), B2M (on average) increased by 0.16 mg/L, non-significant at p=0.33, with unchanged values of other parameters.

Table 1. Multiple regression / sFLC ratio, TP, SPEP & B2M

Regression Summary for Dependent Variable: B2M R= 0,99; F(3,3)=38,979 p<0,007						
N=7	Beta	Std.Err. of Beta	B	Std.Err. of B	t(3)	p-level
Intercept			41,50	8,92	4,65	0,02
sFLC ratio	-1,26	0,16	-0,11	0,01	-8,05	**0,004**
TP (serum)	0,21	0,18	0,16	0,14	1,15	0,33
SPEP	-1,84	0,24	-50,60	6,47	-7,82	**0,004**

Source: Georgievski, O. *Table 1* [picture].

sFLC-ratio, TP, SPEP & ALB (serum)

Between ALB (serum) as a dependent variable & sFLC-ratio, TP, SPEP, as independent variables for R = 0.98 there was a very strong significant (p<0.05) correlation (Table 2). The greatest influence on ALB (serum) had SPEP Beta = 2.05, then TP (serum) Beta = - 1.90, and the weakest was the influence of sFLC-ratio Beta = 0.59. Patients with a pathological finding on SPEP had (on average) 31.34 g/L higher value of ALB (serum) compared to patients who had a normal finding, significant for p=0.007, with unchanged values of other parameters. With each unit value increase in TP (1 g/L), ALB (serum) (on average) decreased by 0.81 g/L, significant at p =0.004, with unchanged values of other parameters.

With each unit value increase in sFLC-ratio, ALB (serum) (on average) increased by 0.03 g/L, non-significant at p=0.07, with unchanged values of other parameters.

Table 2. Multiple correlation / sFLC ratio, TP, SPEP & ALB (serum)

Regression Summary for Dependent Variable: ALB (serum) R= 0,98; F(3,3)=21,324 p<0,02						
N=7	Beta	Std.Err. of Beta	B	Std.Err. of B	t(3)	p-level
Intercept			84,75	6,64	12,77	0,001
sFLC ratio	0,59	0,21	0,03	0,01	2,83	0,07
TP (serum)	-1,90	0,24	-0,81	0,10	-7,89	**0,004**
SPEP	2,05	0,31	31,34	4,82	6,51	**0,007**

Source: Georgievski, O. *Table 2* [picture].

Difference / Study Group & Control Group

B2M value was significantly higher in the study group Z = 3.28 and p=0.001.
sFLC-ratio value was significantly higher in the study group Z = 2.44 and p=0.01 (Table 3).

Table 3. Difference /Study group & Control group / analyzed parameters

Variable	Rank Sum Study	Rank Sum Control	U	Z adjusted	p-level	Valid N	Valid N
B2M	96,50	56,50	1,50	3,28	**0,001**	7	10
sFLC-ratio	88,00	65,00	10,00	2,44	**0,01**	7	10

Source: Georgievski, O. *Table 3* [picture].

4. DISCUSSION

In our research, we evaluated the association of some common laboratory caracteristics of the disease with the basic panel for the diagnosis of MM: sFLC-ratio, TP and SPEP. The association of sFLC-ratio with B2M p=0.004 was primarily the result of the biological quality of these parameters. Namely, their concentration in the blood increases in case of kidney failure, polymerization, but also as a reflection of the size of the tumor mass, which was especially manifested by the elevated concentrations of the immunoglobulin light chains and their pathological ratio result, as well as the elevated concentrations of B2M. B2M belongs to the beta globulin family and is associated (non-covalently bound) with HLA I antigen of nucleated cells, particularly those included in immune response and is included in the cellular defence and malignant cell control (Argyropoulos et al, 2017). MM malignant plasmocytes obtained from biopsy material have been proven to produce B2M and the rate of B2M release is higher in proliferating lymphoid tissue than in quiescent lymphoid cells (Bataille, Grenier & Sany, 1984). At the same time, elevated values for B2M concentrations are associated with unfavorable prognosis (Wallington-Beddoe & Mynott, 2021). But the main drawback of B2M is that it is non-specific for MM and its blood concentrations are influenced by residual kidney function, so it is also a marker of kidney damage (Assounga, 2021).

In the case of the weak correlation of ALB p=0.07 with sFLC-ratio from our results the explanation is probably related to the small number of treated subjects, although our results are confirmed by some of the results obtained from the cited literature. Secondly, the weak association was also result of the stage in which the disease is diagnosed. This is the case with patients at the beginning of the illness, when no general deterioration of the body's metabolic capacities exist. Serum ALB level is considered as a nutritional indicator, disease performance parameter and marker for inflammatory status of the body, so low levels of ALB are important laboratory characteristic of MM (Cai, Zhao, Dai, Xu, Xu & Xia, 2021). It would be expected to establish a significant correlation, if we take into account the significance of sFLC-ratio as a tumor marker in monoclonal gammopathies and the role of albumins in the prognosis of the disease when the disease would be detected at an advanced stage, but this is not the subject of our research.

In the study of Pika T. (Pika et al, 2008) the correlation between sFLC-ratio and the level of B2M in serum was evaluated in terms of kappa and lambda secretors, so it was found that in the group of kappa secretors the association between sFLC-ratio and B2M level was for r = 0.316, p<0.01, and in the group of lambda secretors the association was for r=–0.473, p=0.003.

In Sthaneshwar's study (Sthaneshwar, Nadarajan, Maniam, Nordin & Gin Gin, 2009), a significant correlation of sFLC ratio with B2M was found, p<0.001, but not with ALB, p>0.05. Abnormal initial sFLC ratio values that correlated significantly with B2M indicated a more aggressive form of the disease and a high tumor burden.

In the study by De Novellis (De Novellis et al, 2022) sFLC ratio and B2M correlated significantly (r = 0.7549; p < 0.0001), but no association was found between sFLC ratio with ALB (p = 0.9638).

Kyrtsonis (Kyrtsonis et al, 2007) determined in his study a relationship of sFLC ratio with LDH (p< 0.02) at the initial diagnosis of MM. However, in that research, the existence of a correlation between sFLC ratio and B2M and ALB was not established, which is again in support of Mead's research (2004) (Mead et al, 2004, as cited in

Kyrtsonis et al, 2007). Cavallo (2005) (Cavallo et al, 2005, as cited in Kyrtsonis et al, 2007) confirmed the opposite, correlation between sFLC ratio and B2M.

5. CONCLUSION

From the results of our study, we can confirm the association between sFLC ratio and B2M as an indicator of tumor mass (burden) in multiple myeloma, as well as with the biological characteristics of myeloma cells.

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