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The correlation between serum free light chain levels and plasma cell ratio in bone marrow biopsy in multiple myeloma

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ABSTRACT

Aims: MM (Multiple myeloma) is the second most common hematological malignancy. In addition to the recent advances in treatment, new parameters are used in clinical practice in diagnosis and follow-up. sFLC (free chain kappa and lambda) shows the activation of the disease depending on the rate of MM malignant cell secretion in serum. However, the plasma cell (PC) ratio in bone marrow biopsy is still the gold standard in diagnosis. We examined the dynamic correlation between the PC ratio and the number of sFLC-related cells and the secretion rate. We aimed to examine whether a low PC ratio could be in a more aggressive form with a higher sFLC secretion with too much activity, thus examining the correlation between them.

Methods: A total of 62 newly diagnosed MM patients admitted to Başkent University Faculty of Medicine İstanbul Hospital were included in the study. At the time of diagnosis, sFLC values were requested simultaneously with bone marrow biopsy. Radiological images were obtained with PET CT/MRI or CT.

Results: In all MM groups, bone marrow PH percentages were not correlated with sFLC regardless of subtype. IgG kappa type MM had the highest sFLC values despite the lowest number of PHs, while Lambda light chain MM had the lowest sFLC despite the highest PH rates.

Conclusion: These results showed us that sFLC rates are independent of the percentage of PC in MM. We believe that the two are not correlated and should be followed up together in the follow-up of the disease.

Keywords: Multiple myeloma, bone marrow biopsy, serum free light chains, radiological involvement, plasma cell ratio

INTRODUCTION

Multiple myeloma (MM) is the second most common disease with a rate of approximately 1% among all cancers and 10% among all hematological malignancies.[1,2] It is more common in males than females, with a median age at diagnosis of 65 years.[3] In MM, it is considered that the disease emerges after the MGUS (monoclonal gammopathy unknown significance) and SMM (smoldering myeloma) stages.[4] The diagnostic criteria for all plasma cell diseases such as MGUS, SMM, MM, solitary plasmacytoma, POEMS syndrome and systemic amyloidosis have been clearly defined by the consensus report of IMWG (International Myeloma Working Group).[5]

Demonstration of increased monoclonal protein in a patient with suspected MM is the most important parameter in diagnosis and follow-up. Monoclonal (M) proteins produced by MM malignant plasma cells can be analysed by various methods. Serum protein electrophoresis (SPEP) method, which is still frequently used in clinical practice, is a cheap and easy to access test both in screening and follow-up. However, its disadvantage is that although it determines the presence of M protein in the spike, it does not determine its type. Small increases of M protein in IgD or IgE type MM can be easily overlooked, whereas serum protein elements other than immunoglobulins, such as fibrinogen, may falsely suggest the presence of an M protein.[6,7]

Again, secondary hemoglobin-haptoglobin complexes appearing as a broad band in the alpha-2-globulin region in cases of hemolysis, high transferrin concentrations in patients with iron deficiency anaemia forming a localised band in the beta region, increased alpha-2 and beta bands in nephrotic syndrome are among the reasons that cause confusion in the detection of M protein.

The method used to determine the type of M protein is serum immunoelectrophoresis. The difference of this test is its low sensitivity in determining the type of M protein. For example, HDL, bilirubin, LDL cholesterol, CRP, antistreptolysin-O, creatinine, creatinine, glucose, sodium,

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chloride, bicarbonate, urea nitrogen, albumin, iron and inorganic calcium may interfere with measurements and cause erroneous test results.

In recent years, important steps have been taken in the diagnosis and follow-up of MM. Quantitative analyses of serum free light chain (sFLC=free light chain) Kappa and Lambda are accepted as indicators of plasma cell secretion both in diagnosis and follow-up. (FLC) analysis is a sensitive antibody-based system that detects low concentrations of monoclonal FLC (i.e. kappa or lambda) in serum. This method is also important in that it shows monoclonal proteins at concentrations too low to be detected by routine serum immunofixation techniques in approximately 16% of MM patients who produce only Bence Jones protein (FLC not linked to a heavy chain).[8] Normal values of serum free light chains are shown in **Table 1**.[9]

Table 1. Normal values of serum free light chains and ratio

Free serum kappa light chains - 3.3 to 19.4 mg/L
Free serum lambda light chains - 5.7 to 26.3 mg/L
The ratio of kappa to lambda FLCs - 0.26 to 1.65

However, in patients with renal impairment, serum FLC concentrations increase with a decrease in glomerular filtration rate as a result of the normally rapid renal clearance of serum FLCs in the presence of renal impairment (e.g., creatinine clearance <60 mL/min) and may reach values 20 to 30 times normal in end-stage renal failure.[10] Conflicting results have been obtained in studies evaluating the kappa/lambda ratio in patients with renal failure. Some studies show that the ratio is increased in patients with severely reduced renal function.[11] For example, one study showed that the kappa/lambda ratio, which normally ranges from 0.26 to 1.65, can be as high as 3.1 in the presence of renal failure due to dialysis.

Morphological Appearance

The morphological characteristics of plasma cells may differ depending on their maturity and are sometimes morphologically indistinguishable from myeloblasts. Mature plasma cells are oval and have abundant basophilic cytoplasm. The nucleus is round and eccentrically located with a prominent perinuclear hoph or cytoplasmic clearing. The nucleus contains "clock face" or "spoke" chromatin without nucleolus. Plasma cells observed in myeloma vary from mature forms to immature (**Figure 1A**), plasmablastic (**Figure 1B**) and pleomorphic types. Immature plasma cells have scattered nuclear chromatin, prominent nucleoli, and a high nuclear/cytoplasmic ratio. Approximately 10% of cases contain plasmablastic morphology.[12] In some cases, multinuclear, multilobed and pleomorphic plasma cells predominate. The cytoplasm of myeloma cells containing dense endoplasmic reticulum may contain condensed or crystallised cytoplasmic immunoglobulin, resulting in the following unusual findings not limited to MM: Numerous pale bluish-white, grape-like deposits (Mott cells, Morula cells), cherry-red refractive round bodies (Russell bodies), glycogen-rich IgA (Flame cells), Vermillion-stained overfilled fibrils (Gaucher-like cells, tseaurocytes) and crystal rods.

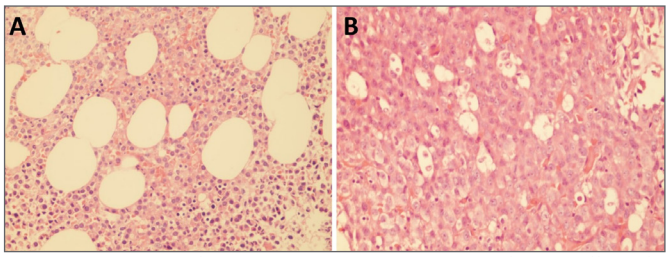

Figure 1. Hematoxylin-Eosin stainx400: Immature plasma cells with eccentric nuclei (A). Hematoxylin-Eosin stainx400: Plasmablastic morphology with distinct nucleoli (B)

Immunophenotype

Immunohistochemical staining, immunofluorescence studies and flow cytometry can be used to determine the immunophenotype of bone marrow plasma cells in patients with MM. The normal kappa/lambda ratio in bone marrow is 2:1. A ratio of more than 4:1 or less than 1:2 is considered to fulfil the definition of kappa or lambda monoclonality, respectively. This finding distinguishes monoclonal gammopathies from reactive plasmacytosis. Neoplastic plasma cells contain Kappa or Lambda monotypic cytoplasmic Ig and usually lose superficial Ig. Myeloma cells express paler CD38 and brighter CD138 than normal plasma cells. In contrast to normal plasma cells, myeloma cells are CD45 negative or expressed at very low levels. CD19, which is usually positive in normal plasma cells, is negative in 95% of myeloma cells. Some antigens, which are absent or detected at very low rates in normal plasma cells, are seen in approximately 90% of neoplastic plasma cells. Among these antigens, CD56 is expressed 75-80% and CD 200 is expressed 60-75% in myeloma cells.[13-15]

METHODS

Our study was approved by Başkent University Medical and Health Sciences Researches Ethics Board (Date: 12.12.2023, Decision No: KA-23408 /12.12.2023). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 62 newly diagnosed MM patients who were admitted to the Hematology Clinic of Başkent University Medical Faculty İstanbul Hospital in the last 5 years were included in the study. Patients were classified as IgG K/L IgA K/L IgD IgM and light chain kappa /Lambda according to the type of MM. The patients included in the study were diagnosed with MM according to the criteria

specified by IMWG. MGUS and Smoldering Myeloma (SMM), AL amyloidosis cases were excluded from the study. In the retrospective analyses of the patients, sFLC values and sFLC k/l ratios at the time of diagnosis were compared with the plasma cell percentages detected in the pathology samples obtained from the bone marrow at the time of diagnosis. Findings in favour of bone involvement in any of the radiological imaging studies (PET, MRI or CT) were accepted as bone involvement.

Statistical Analysis

Statistical analyses were performed using "IBM SPSS Statistics for Windows. Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)". Spearman correlation test was used to examine the correlation between continuous variables that were not normally distributed. $p<0.05$ was considered statistically significant.

RESULTS

A total of 62 patients (27 (43.55%) males and 35 (56.45%) females) with MM were included in the study. The mean age of female patients was 65.53 years and the mean age of male patients was 68.62 years. The mean age was 67.07 years in the whole group. The youngest patient was female at the age of 30.8 years and the oldest patient was male at the age of 90.3 years. Bone involvement was present in both patients at the time of diagnosis. Bone involvement was 67.74% (42 patients) in the whole group. This rate was 74% (20 patients) in the male patient population and 62.85% (22 patients) in the female patient population (**Table 2**).

Table 2. Demographic data

	Male	Female
Total number of patients (n=62)	27 (43.55%)	35 (56.45%)
Average age	68.62	65.53
bone involvement	27/20 (74%)	35/22 (62.85%)
IgG kappa MM (n=45)	n=21	n=42
IgG lambda MM (n=9)	n=3	n=6
IgA lambda MM (n=1)	n=1	n=0
Kappa light chain MM (n=4)	n=1	n=3
Lambda light chain MM (n=3)	n=1	n=2

IgG-Kappa MM was detected in 45 (72%) of the patients included in the study. Among the patients, 21 were male and 24 were female and bone marrow involvement was present in 60% of this group. The number of patients diagnosed with IgG-Lambda MM was 9 in total, 3 males and 6 females. Bone marrow involvement was found in 88.8% (8 patients). IgA-Kappa MM was observed in 1.6% (1 patient, bone marrow involvement positive), Kappa light chain MM in 6.4% (1 male, 3 female patients; bone marrow involvement positive in 3 patients), Lambda light chain MM in 4.8% (1 male, 2 female patients; bone marrow involvement positive in all). PC ratios of the patients according to MM types are shown in **Table 3**. The correlation between sFLC ratios and plasma cell percentages is shown in the **Table 4**.

Table 3. Plasma cell ratios and sFLC values according to MM types

MM Types	Mean % Plasma Cells	Mean S FLC Kappa/Lambda
IgG kappa MM	37.28%	104.201
IgG lambda MM	44.3%	113.699
IgA kappa MM	70%	30.30
Kappa light chain MM	62.5%	29.2
Lambda light chain MM	60%	139.65

Table 4. Correlation between sFLC ratios and plasma cell percentages

		sFLC/k	sFLC/L	K/L ratio	Diagnosis PC%
sFLC/k	r	1			
	p				
sFLC/L	r	-0.057	1		
	p	0.658			
K/L ratio	r	0.251*	0.119	1	
	p	<0.05	0.356		
Diagnosis PC%	r	0.017	0.058	0.122	1
	p	0.893	0.653	0.343	

*p<0.05; Spearman correlation test: As seen in Table 4, it is possible to say that there is a statistically positive correlation between sFLC/k variable and K/L Ratio (p<0.05). Accordingly, it can be said that as the sFLC/k value increases (or decreases), the K/L Ratio value also increases (decreases). However, no correlation was found between serum free kappa and free Lambda and bone marrow per cent plasma cells.

DISCUSSION

Since the free FLC value to be applied in our study will directly show the pathologically increased PC clone, we aimed to reveal the correlation between the rate of malignant plasma cell clone and free light chains secreted from the clone in our study. Thus, we investigated the correlation of bone marrow PC rates and differences with sFLC.

Due to the monoclonal nature of MM, only one of the kappa or lambda light chain is increased as a monoclonol. The plasma cell prefers light chain production to heavy chain production, which requires less ATP and can be secreted quickly and rapidly. Thus, the initial increase of the light chain before the start of heavy chain production is considered more appropriate for early detection of the rapidly changing character of the disease. With this feature, it is very valuable in the diagnosis and progression monitoring of patients with non-secretory MM and oligosecretory (monoclonal protein in serum <1 g/dL [10 g/L] MM and monoclonal protein in urine <200 mg/day), AL amyloidosis as well as light chain myeloma. Predicting the risk of progression of MGUS. In the follow-up of smoldering MM. Their use is important in the progression of solitary plasmacytoma of bone.[16-18]

MM is a highly heterogeneous disease with different genetic and molecular mechanisms. This different nature of the disease leading to differences during the course

of the disease was demonstrated by B. Barlogie at the Arkansas Myeloma Institute (UAMS) with a classification based on PCR-based gene expression profiling.[19] Even if the patient has the same genetic structure and the same type of MM, it is possible that they may present in different ways. This is due to differences in expression rates and expressed proteins from the underlying pathological gene. Therefore, this heterogeneous group requires a test that is sensitive to the course of the disease and that can rapidly show instantaneous changes, This test is a quantitative measurement of free kappa and lambda in serum and their ratio to each other. The important point that should not be overlooked here is that even if the free chain amounts are normal, the proportional distortions between them may be pathological and therefore may be a very early indicator in disease follow-up.

The gold standard test that we still use in current hematology practice at the time of diagnosis and in case of nuclei is the plasma cell count in bone marrow biopsy and the percentage of these cells in the bone marrow. However, some questions arise here. Is it necessary to increase the PH number quantitatively in order to increase the M protein? Can the secretion rate of PC increase or decrease regardless of the PC rate? Which parameter should be prescribed for treatment in this case? Which should be taken into account in assessing both nucleus and remission status?

Although plasma cell percentage is still a major parameter among the diagnostic criteria for MM, we sometimes encounter unexpectedly high sFLC values due to low plasma cell percentage or vice versa, low serum FLC values despite high PH count. In this case, the question that comes to mind is whether there may be differences in the secretion rate of malignant cells among themselves regardless of the plasma cell ratio or the fact that secretion rates may vary. Thus, a higher proportion of plasma cell numbers may result in less FLC secretion, whereas conversely a lower plasma cell number may have a higher secretory potency. This may affect the complications and survival of the disease. Thus, perhaps a lower proportion of plasma cells may be more destructive. In conclusion, the question of which parameter should be considered is important. Another important point is the MRD (minimal residual disease) tests that we still use in the clinic. The basis of these tests is the quantitative quantification of plasma cell counts in the bone marrow by flow cytometry or PCR-based methods. If plasma cell secretion is independent of the count, the reliability of these tests and MRD may need to be reviewed.

The MM subgroup analysis of the patients in our study was similar to IgG Kappa, which is the most common type in the world. There were more female patients and bone involvement was present in most of the patients.

In our review of the literature, we did not find any study showing a correlation between PH rate and FLC, except for a study conducted in China with a small case group using total FLC.[20] In this study, PH and FLC values were found to be correlated only in Ig G MM and no correlation was found in other MM subtypes. However, total FLC was used in the study and free values were not analysed. This test is not as useful as free FLC, but it is considered to be a very useful test because it is easy to be affected by infection and similar inflammatory processes that are currently increased in the body for other reasons.

In our study, IgG kappa group MM patients were found to secrete the highest sFLC at the lowest cell rate according to the current PC percentages, whereas we found the lowest sFLC values in Lambda light chain patients despite high PC rates.

As a result of our study, we found that there was no correlation between the percentage of plasma cells in the pathologically detected bone marrow biopsy sample and serum free kappa and lambdas. These results suggest that the secretion rate and activity of plasma cell may be independent of the number.

While a low PH number may cause MM to be more aggressive with a high secretion rate, the opposite may be the case. The important point here is that PC is actually in a dynamic process independent of its number, suggesting the possibility that its secretions may increase or decrease in certain periods of the disease.

CONCLUSION

We believe that MM may be better reflected not only by blood and urine M proteins or bone marrow PH count but also by both together. Studies with large patient series including remission and relapse groups with frequent bone marrow biopsies to be performed throughout the course of the disease will be able to define the correlation between PC rate and sFLC more clearly. We believe that the results of these studies may lead to a revision of the diagnostic and remission criteria and MRD concepts.

ETHICAL DECLARATIONS

Ethics Committee Approval

Our study was approved by Başkent University Medical and Health Sciences Researches Ethics Board (Date: 12.12.2023, Decision No: KA-23408 /12.12.2023).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Financial Disclosure
The authors declared that this study has received no financial support.

Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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