Original Article

Simultaneous Evaluation of Serum Immunofixation Electrophoresis and Serum Free Light Chain Measurements Used in the Diagnosis of Monoclonal Gammopathy and Smoldering Multiple Myeloma of Uncertain Significance in the Presence of Risk Factors

Risk Faktörleri Varlığında Önemi Belirsiz Monoklonal Gammopati ve Smoldering Multiple Myeloma Tanısında Kullanılan Serum İmmünfiksasyon Elektroforezi ve Serum Serbest Hafif Zincir Ölçümlerinin Eş Zamanlı Değerlendirilmesi

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ABSTRACT

Introduction: This study aimed to investigate the effectiveness of using serum Free Light Chain (sFLC) together with traditional examination methods in diagnosing Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Multiple Myeloma (SMM), especially in patients with Monoclonal Gammopathy (MG) without hypercalcemia, renal dysfunction, anemia, and bone lesions (CRAB) symptoms, by eliminating the need for bone marrow biopsy (BMb).

Methods: A total of 160 patients over 50 years of age with an ESR of 50 mm/h and above were included in the study. Serum Immunofixation Electrophoresis (sIFE) and sFLC levels of these patients were studied simultaneously. Sensitivity and specificity of the sIFE and sFLC for diagnosis of MGUS were estimated by ROC analysis.

Results: MG was detected in 36 (22.5%) patients with sIFE and in 30 (18.7%) patients with sFLC of 160 patients included in the study.There were a total of 44 patients with MG detected by sIFE alone, sFLC alone, and both sIFE and sFLC. BMb were performed on 42 patients with MG who approved the BMb procedure. These patients were diagnosed MGUS, SMM and MM by using test results, BMb results, and clinical and laboratory data. The sensitivity of sIFE in the detection of MG was 82.5% and the specificity was 99.2% ($p<0.001$). sFLC had a sensitivity of 72.5% and a specificity of 99.2% ($p<0.001$). When sIFE and sFLC were performed simultaneously, the sensitivity was 100% and the specificity was 98.3% ($p<0.001$).

Discussion and Conclusion: Simultaneous monitoring of sIFE and sFLC, which is both non-invasive and with the opportunity to achieve results in less time without BMb, may be sufficient and reliable in the diagnosis and follow-up of MGUS in the detection of MGs and especially in asymptomatic patients.

Keywords: MGUS, ESR height, sFLC

ÖZET

Giriş ve Amaç: Bu çalışmada, özellikle hiperkalsemi, böbrek fonksiyon bozukluğu, anemi ve kemik lezyonları (CRAB) semptomları olmayan Monoklonal Gammopatili (MG) hastalarda, Önemi Belirsiz Monoklonal Gammopati (MGUS) ve Smoldering Multipl Miyelom (SMM) tanısında, serum Serbest

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Hafif Zincirinin (sFLC) geleneksel tetkik yöntemleri ile birlikte kullanımının kemik iliği biyopsisi (Kİb) ihtiyacını ortadan kaldırarak etkinliğinin araştırılması amaçladık.
Yöntem ve Gereçler: Çalışmaya ESR 50 mm/h ve üzerinde olan, 50 yaş üstü 160 hasta alındı. Bu hastaların serum İmmünfiksasyon Elektroforezi (sIFE) ve sFLC düzeyleri eş zamanlı çalışıldı. MGUS tanısı için sIFE ve sFLC'nin duyarlılığı ve özgüllüğü ROC analizi ile tahmin edildi.
Bulgular: Çalışmaya alınan160 hastanın yapılan sIFE ile 36(%22,5) hastada ve sFLC ile de 30 (%18,7) hastada MG tespit edilmiştir. Yalnızca sIFE, yalnızca sFLC ve hem sIFE hem de sFLC tarafından saptanan MG'li toplam 44 hasta vardı. MG tespit edilen ve kemik iliği biyopsi (Kİb) işlemine onay veren 42 hastaya Kİb yapılmıştır. Bu hastalara; test sonuçları, Kİb sonuçları, klinik ve laboratuar verileri de kullanılarak MGUS, SMM ve MM tanısı konulmuştur. sİFE'nin MG tespitinde sensitivitesi %82.5, spesifitesi %99.2 ($p<0.001$) olarak saptanmıştır. sFLC'nin sesensitivitesi %72.5, spesifitesi %99.2 ($p<0.001$) olarak saptanmıştır. sİFE ve sFLC eş zamanlı yapıldığında ise sensitivitesi %100, spesifitesi %98.3 ($p<0.001$) olarak saptanmıştır.
Tartışma ve Sonuç: MG'lerin tespitinde ve özellikle asemptomatik hastalarda Kİb yapmadan hem noninvaziv hem de daha kısa sürede sonuç alma fırsatı ile sIFE ve sFLC'nin eşzamanlı bakılması MGUS tanısı koymada ve takip etmede yeterli ve güvenilir olabilir.

Anahtar kelimeler: MGUS, ESR yüksekliği, sFLC

Introduction

Monoclonal Gammopathies (MG) are a group of diseases characterized by uncontrolled proliferation and accumulation of clonal plasma cells capable of synthesizing light and heavy chains of immunoglobulin (Ig), called monoclonal protein (M protein), which can be detected in serum or urine [1, 2]. MGs range from benign Monoclonal Gammopathy of Undetermined Significance (MGUS) to Smoldering Multiple Myeloma (SMM), which usually does not require treatment and has a silent course, to the highly malignant Multiple Myeloma (MM), which requires treatment [3, 4].

MGUS is a MG that is more common in the elderly and is characterized by mature plasma cell proliferation in the bone marrow (BM) [5]. It is seen at a rate of 3.2% above the age of 50, and at a rate of 5.3% above the age of 70 [6]. Its prevalence increases with age and this rate reaches 8.7% over the age of 80 [7-9].

MGUS may also include a heterogeneous group such as SMM, which is an intermediate stage of the disease as it progresses to MM. This group may exhibit biological behaviors similar to MGUS, but may also include clinical manifestations such as hypercalcemia, renal dysfunction, anemia, and bone lesions (CRAB symptoms) requiring treatment for myeloma [10]. MM is a group of malignant diseases characterized by neoplastic transformation of plasma cells and clonal growth of B cells in BM [1, 2].

Comprehensive clinical and laboratory evaluation is required to differentiate MGUS, SMM, and MM patients. It is important to make this distinction. Because while active treatment is required in MM, follow-up is more important in MGUS and SMM due to the risk of progression to MM rather than treatment.

Excessive and disproportionate production of kappa or lambda free light chains occurs in MGUS or MM [11]. Therefore, measurement of free light chain levels in serum has an important place in the diagnosis of diseases that cause abnormal monoclonal or polyclonal light chain concentrations.

Traditional methods such as serum Protein Electrophoresis (sPE), serum immunofixation electrophoresis (sIFE), and nephelometric measurement of serum Ig heavy chains are used in the diagnosis and follow-up of MGs [12)]. However, the lack of standardization,

limited sensitivity and specificity of these methods are the main limitations [13, 14].

With the introduction of serum free light chain (sFLC) measurement method [15], it has gained an important place in hematological diagnoses by changing the diagnostic procedures [16-18]. Detection of sFLC is very useful in the diagnosis, follow-up and prognosis of MGs [19]. There are also studies showing that sFLC is a reliable marker that can be used together with conventional tests in the detection and follow-up of MGs [20].

MGUS was updated again in 2014 to include the criteria used in the differential diagnosis of SMM and MM in the International Myeloma Working Group (IMWG) guide [18] in the sFLC. The diagnostic criteria of these diseases include the demonstration of clonal plasma cell proliferation by BMb [18, 21-23].

In this study, we aimed to investigate the effectiveness of using sFLC together with traditional examination methods in diagnosing MGUS and SMM, especially in patients with MG without CRAB symptoms, by eliminating the need for BMb, which is an invasive procedure, in the presence of certain risk factors.

Materials and Methods:

The study was planned as a prospective study. The study protocol was prepared in accordance with the Declaration of Helsinki and was accepted by the Erciyes University Faculty of Medicine Ethics Committee (Ethics Committee Decision No: 2022/340).

Patients:

Between January 2012 and January 2013, 160 patients who were followed up in with an ESR 50 mm/s and above, over 50 years of age, with bonepain and informed consent form were included followed in the Haematology clinic of Erciyes University Faculty of Medicine. Those below the age of 50 and with ESR below 50 mm/s, plasma cell disorders, other lymphoproliferative diseases, rheumatoid arthritis, Sjogren's, psoriatic arthritis vs soft tissue diseases, infections such as hepatitis C, HIV, scleroderma, urticaria vs dermatological diseases and autoimmune diseases were excluded from the study.

Laboratory:

The serum samples of the patients included in the study were collected in the immunology laboratory for sPE, sIFE and sFLCtests. sIFE, sFLC kappa and lambda from the serums were stored at -20°C to be measured. Completeblood count, biochemical tests (serum calcium, phosphorus, total protein,albumin, creatinine) levels were studied. BMb samples were taken from the patients with a peak in the gamma band in sPE and MG detected in sIFE and sFLC tests, and both microscopic evaluation and histopathological diagnosis were made. Patients who did not accept BMb were evaluated clinically and with other laboratory parameters.

The sPE agarose gel method was studied on a semi-automated SAS-1Plus/SAS-2/Platinum device (Helena Biosciences Europe, Tyne and Wear, England). BMb was planned for patients with sPE and M protein levels of 1.5 g/dL and above. sIFE was performed using the interlabG26 Agarose Gel Electrophoresis Analyzer and the Slide method Paragon IFE kit (Beckman Coulter, Brea, CA). The sensitivity for IFE varied between 50-150 mg/dL depending on the type of monoclonal protein. Interpretation in sIFE was made visually and qualitatively. Measurement of sFLC was performed on an automated nephelometry instrument (BN ProSpec, Dade, Germany) with reagent (Freelite™, The Binding Site Ltd, Birmingham, UK). For each test, a standard dilution of 1/100 was run as specified by the manufacturer. When "antigen excess" was detected, the measurement was repeated with a higher dilution (1/400 and 1/2000).

Statistical Analysis:

Statistical analyzes were performed with IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Clinicopathological variables of patients associated with MG were evaluated for normal distribution using the Kolmogorov Smirnov test and visual methods. In descriptive statistics, parametric continuous variables were presented as mean (standard deviation), nonparametric variables were presented as median (range), and categorical variables were presented as frequency (percentage). According to the normal distribution status, numerical data were compared between groups with Student's T test or Mann Whitney U test, and categorical data were compared between the groups with Chi-square test or Fisher's exact test according to their suitability. AUC, cut-off values, sensitivity and specificity of the tests used to diagnose MG were evaluated by ROC analysis. Comparison of the areas under the curve of these tests was done by pairwise comparisons. Statistically $p<0.05$ was considered significant.

Results

Both sIFE and sFLC levels of 160 patients included in the study were evaluated. MG was detected in 36 patients (22.5%) with sIFE. MG was detected in 30 patients (18.7%) with sFLC. A total of 44 patients with MG detected by only sIFE, only sFLC, both sIFE and sFLC were recommended to undergo BMb, and BMb could not be performed in two patients with MG in sIFE, since they did not give voluntary consent. MGUS, SMM, MM were diagnosed by looking at the plasma cell percentages and clinics of 40 of the 42 patients who underwent BMb, and the other two biopsy results were determined as B-cell lymphoma and MDS, respectively. MG was detected by the sIFE method in 34 of these patients, and the diagnostic distribution was found to be MGUS 24 patients, SMM three patients, MM six patients, and one other (MDS). Again, in 30 of these patients, MG was detected by the sFLC method, and the diagnostic distribution was found to be MGUS 21 patients, SMM three patients, MM five patients, and one other (B-cell lymphoma). (Figure.1)

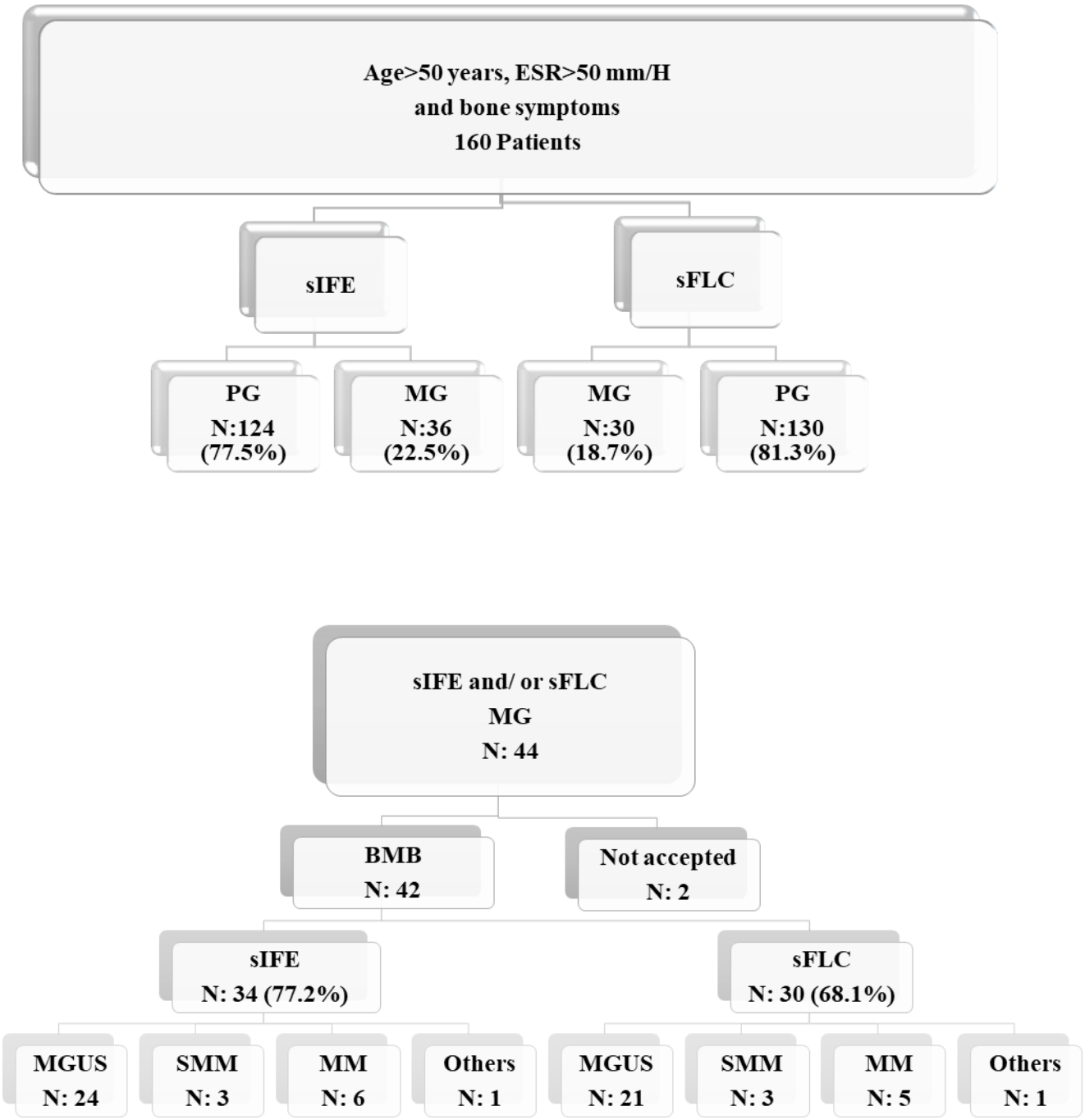
A comparison of 44 patients with MG with sIFE or sFLC was compared with the remaining 116 patients with polyclonal gammopathy (PG) (Table 1). There was a statistically significant difference between the two groups in terms of gender, and the rate of male patients was found to be higher in the MG group (P=0.001).

Patients were evaluated in terms of CRAB parameters included in the MM diagnostic criteria. Hypercalcemia was observed in 15.9% of the patients with MG, while it was observed in 0.9% of the PG patient group (p=0.001). In terms of osteoporosis, when the two groups were compared, osteoporosis was found in 46.3% of the MG patients, while osteoporosis was found in 16.7% of the PG group (p=0.030). When the two groups were compared in terms of laboratory parameters; A statistically significant difference was found in terms of IgA, IgM, sIFE lambda, BUN, creatinine and T score levels. While the median age of the patients with MG was 67.5 (range:60.5-73.5 years), the median age of the PG group was 64.0 (range:56.0-73.0 years, p=0.143) (Table.2).

Descriptive statistics of ROC curve via sIFE, sFLC, sIFE or sFLC according to MG detected in bone marrow biopsy was shown in Table3.

When the area under the curve in the ROC curves was compared for these three methods, no statistically significant difference was found between measuring only sIFE and only sFLC (p=0.350, Table 4). When sFLC was evaluated together with sIFE, it was found that detecting patients with MG was statistically significantly superior to measuring sIFE alone or sFLC alone (p=0.007; p<0.001, respectively) (Figure.2).

Figure 1: Diagnostic Approach Results According to Immunological Tests and Bone Marrow Biopsy



ESR: Erythrocyte Sedimentation Rate, sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, PG: Polyclonal Gammopathy, MG: Monoclonal Gammopathy, BMB: Bone Marrow Biopsy, MGUS: Monoclonal Gammopathy of Undetermined Significance, SMM: Smoldering Multiple Myeloma, MM: Multiple Myeloma

Table 1: Comparison of Categorical Data Between Two Groups

		Total N=160	PG N=116	MG N=44	p value
Gender	**Female**	96 (60%)	79 (68.1%)	17 (38.6%)	0.001
	Male	64 (40%)	37 (31.9%)	27 (61.4%)	
Age	**50-69 years**	10 (63.8%)	75 (64.7%)	27 (61.4%)	0.699
	70 year and over	58 (36.3%)	41 (35.3%)	17 (38.6%)	
Diagnosis According to sIFE	**Polyclonal Gammopath**	124 (77.5%)	116 (100%)	8 (18.2%)	
	Ig G Kappa (mg/mL)	19 (11.9%)	0 (0%)	19 (43.2%)	
	Ig G Lambda (mg/mL)	13 (8.1%)	0 (0%)	13 (29.5%)	
	Ig A Kappa (mg/mL)	4 (2.5%)	0 (0%)	4 (9.1%)	
MG Status (According to sIFE)	**No**	124 (77.5%)	116 (100%)	8 (18.2%)	<0.001
	Yes	36 (22.5%)	0 (0%)	36 (81.8%)	
Diagnosis According to sFLC	**Normal serum**	26 (16.3%)	21 (18.1%)	5 (11.4%)	
	MG	19 (11.9%)	0 (0%)	19 (43.2%)	
	MG with RF	11 (6.9%)	0 (0%)	11 (25%)	
	Polyclonal Ig Increase or RF	104 (65%)	95 (81.9%)	9 (20.5%)	
MG Status (According to sFLC)	**No**	130 (81.3%)	116 (100%)	14 (31.8%)	<0.001
	Yes	30 (18.8%)	0 (0%)	30 (68.2%)	
eGFR	**< 60 (mL/dk/1.73m²)**	34 (21.3%)	21 (18.1%)	13 (29.5%)	0.114
	≥ 60 (mL/dk/1.73m²)	126(78.8%)	95 (81.9%)	31 (70.5%)	
Distribution of Diagnoses According to BMB	**MGUS**			31 (64.6%)	
	SMM			3 (6.3%)	
	MM			6 (12.5%)	
	Other			2 (4.2%)	
	None accept			2 (4.2%)	
Corrected Calcium	**>11 (mg/dL)**	8 (5%)	1 (0.9%)	7 (15.9%)	0.001
	≤ 11 (mg/dL)	152 (95%)	115(99.1%)	37 (84.1%)	
R F	**eGFR <40 (mL/dk/1.73m²)**	13 (8.1%)	9 (7.8%)	4 (9.1%)	0.754
	eGFR≥ 40 (mL/dk/1.73m²)	147(91.9%)	107(92.2%)	40 (90.9%)	
Anemia (Female <12; Male <13)	**Yes**	119(74.4%)	87 (75%)	32 (72.7%)	0.769
	No	41 (25.6%)	29 (25%)	12 (27.3%)	
Osteoporosis	**T score < -2.5 (osteoporosis)**	22 (37.3%)	3 (16.7%)	19 (46.3%)	0.030
	T score ≥ -2.5 (normal)	37 (62.7%)	15 (83.3%)	22 (53.7%)	

PG: Polyclonal Gammopathy, MG: Monoclonal Gammopathy, sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, RF: Renal Failure, Ig: Immunoglobulin eGFR: estimated Glomerular Filtration Rate, BMB: Bone Marrow Biopsy, MGUS: Monoclonal Gammopathy of Undetermined Significance, SMM: Smoldering Multiple Myeloma, MM: Multiple Myeloma

Table 2: Comparison of Groups According to Laboratory Parameters

	Total (N=160)	PG (N=116)	MG (N=44)	P value
Age, years	66 (57-73)	64 (56-73)	67,5 (60,5-73,5)	0.143
ESR	66,5 (54-86)	68,5 (55-86,5)	59 (52,5-80)	0.095
sIg G (mg/mL)	1660 (1390-2125)	1600 (1390-1990)	1820 (1370-2605)	0.077
sIg A (mg/mL)	323 (209-441)	356 (278-462)	100,6 (50,1-290,5)	<0.001
sIg M (mg/mL)	92,8 (49,3-140,5)	110,5 (68,9-154)	41,5 (23,1-85,2)	<0.001
sIg E (mg/mL)	26,2 (17,3-100,8)	28 (17,3-135)	17,3 (17,3-71,4)	0.219
sIFE Kappa (mg/L)	405,5 (331-499,5)	400 (341,5-488,5)	441,5 (243-655)	0.828
sIFE Lambda (mg/L)	220,5 (163,5-297,5)	235,5 (182,5-291)	193,5 (63,4-345,5)	0.043
sIFE Kappa/Lambda	1,8 (1,5-2,3)	1,8 (1,5-2)	2,8 (0,9-9,4)	0.093
sBeta 2 Microglobulin (µg/mL)	3,1 (2,5-4,3)	3,1 (2,5-4,1)	3,4 (2,6-5,3)	0.094
sFLC Kappa (mg/L)	34 (20,8-54,1)	33,8 (21,8-51,1)	36,7 (14,1-97,6)	0.577
sFLC Lambda (mg/L)	42,3 (22,4-82,9)	42,7 (28,6-79,2)	39,8 (14,1-93,2)	0.396
sFLC Kappa/Lambda	0,8 (0,6-1,1)	0,8 (0,6-0,9)	1,1 (0,2-2,7)	0.143
Calcium Level (mg/dL)	9 (8,6-9,4)	9 (8,6-9,4)	9 (8,6-9,5)	0.757
Corrected Calcium (mg/dL)	9,6 (9,2-10)	9,6 (9,2-10)	9,6 (9,2-9,9)	0.942
Hemoglobin (g/dL)	11,2(10,2-12,2)	11,2(10,3-12,2)	11,1(9,7-12,2)	0.448
Total Protein (g/L)	7,3(6,8-7,7)	7,2(6,9-7,7)	7,4(6,7-8)	0.431
Albumin (g/L)	3,3(2,8-3,7)	3,3(2,8-3,7)	3,4(2,9-3,8)	0.997
BUN (mg/dL)	18(13-26,5)	17(13-24,5)	21(17-29,5)	0.028
Creatinine (mg/dL)	0,7(0,6-1,1)	0,7(0,6-0,9)	0,9(0,7-1,3)	0.002
T score (BMD)	-2,3(-2,7--1,8)	-2(-2,4--0,9)	-2,4(-2,8--2)	0.019
eGFR (mL/dk/1.73m²)	89.5(8.5-179.1)	91.8(9-179)	87(8.5-146)	0.108

PG: Polyclonal Gammopathy, MG: Monoclonal Gammopathy, ESR: Erythrocyte Sedimentation Rate, sIg: serum Immunoglobulin, sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, BMD: Bone Mineral Densitometry, eGFR: estimated Glomerular Filtration Rate

Table 3. Descriptive Statistics of ROC Curve According to MG Detected in Bone Marrow Biopsy

	Sensitivity(%)	Spesificity(%)	*p* value	AUC	95% CI	LR(+)	LR(-)
sIFE	82.5	99.2	<0.001	0.908	0.852-0.948	97.4	0.2
sFLC	72.5	99.2	<0.001	0.858	0.794-0.909	85.6	0.3
sIFE or sFLC	100	98.3	<0.001	0.992	0.962-1,000	59	0.0

ROC:Receiver Operator Characteristic, MG: Monoclonal Gammopathy, sIFE:Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, AUC: Area Under The Curve, CI: Confidence Interval, LR(+): Likelihood Ratio For a Positive Result, LR(-) :Likelihood Ratio For a Negative Result

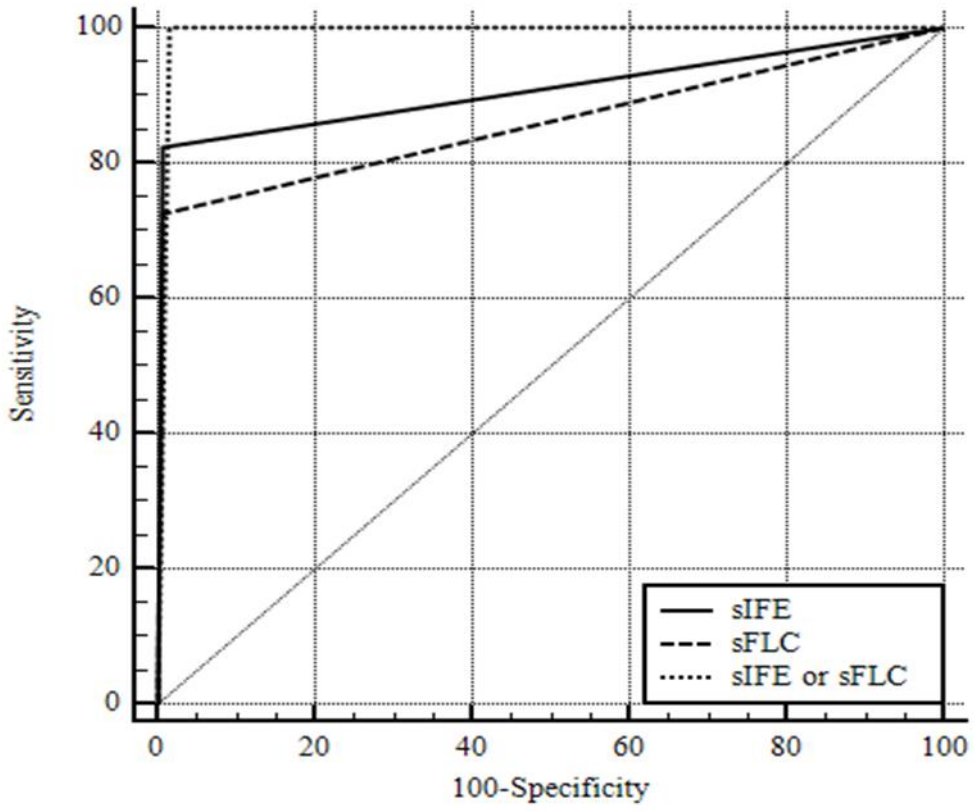


Figure 2: Comparison of ROC Curves

sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain

Table 4. Pairwise Comparison of ROC Curves

	Difference Between Areas	SE	95% CI	z statistics	*P*-value
sIFE-sFLC	0.050	0.053	-0.055-0.155	0.935	0.350
sIFE-sIFE or sFLC	0.083	0.031	0.023-0.143	2.711	0.007
sFLC-sIFE or sFLC	0.133	0.036	0.063-0.204	3.702	<0.001

ROC: Receiver Operator Characteristic,sIFE: Serum Immunofixation Electrophoresis, sFLC: Serum Free Light Chain, SE: Standard Error, CI: Confidence Interval

Discussion

The present study aimed to detect MGs in the presence of risk factors such as high ESR, bone pain and advanced age, and to diagnose MGUS, SMM, and MM among them. At the same time, our aim is to compare the consistency of the results between sIFE and sFLC measurement tests used in the detection of MGs, to determine which method is more sensitive in diagnosing MGUS, and to show whether serum free light chain (κ and λ) measurements can be an alternative to the sIFE method, which is routinely applied in the Immunology laboratory, and which one is more sensitive in detecting MG. While determining the sensitivity and specificity of the sPE, sIFE and sFLC tests, we aimed to diagnose MGUS and SMM in patients with MG without the need for BMb.

Apart from traditional methods such as sPE and sIFE, there are many studies showing that sFLC has an important role in diagnosis, follow-up and progression of MGUS [16, 18-20, 24]. There are many studies that found that κ/λ measurement with the sFLC method is an important predictor of the progression from MGUS to MM in patients with MG [24, 25]. However, sFLC measurements have been shown to be better than sPE and sIFE in early detection of disease progression [16, 26].

Apart from these methods, evaluation of BMb plays a key role in the differentiation of MGUS patients into SMM and MM [18, 21-23]. In the IMWG guideline, BMb is also included among the diagnostic criteria for MGUS, SMM, and MM from MGs [27].

On the one hand, sFLC shows the variation in the concentration ratio of the two chains without direct evidence for the presence of a monoclonal light chain, while sPE/sIFE reveals the true presence of a monoclonal immunoglobulin or monoclonal light chain, thus suggesting that it is a better test than sFLC [28].

In this study, we diagnosed MG in 22% of patients with sIFE and 18.7% with sFLC. We performed BMb, which we accept as the gold standard method, in 42 of 44 patients with MG detected by both methods. We detected plasma cell percentages in 40 of these patients. Thus, we found the sensitivity of sIFE as 82.5% and of sFLC as 72.5% in detecting MG. Therefore, while sIFE found 19% of patients with MG detected by BMb to be normal, we found 28.5% of these patients to be normal with sFLC.

Beetham et al. reported the specificity of the abnormal FLC κ/λ ratio as 96% and the sensitivity as 76% in patients with MG in a

study conducted by [29]. Jaskowski et al. compared serum IFE and FLC values in 483 cases and found the specificity of the κ-FLC test as 91.4%, the sensitivity as 99.5% and the agreement as 94.6%, the specificity of the λ-FLC as 99.7%, the sensitivity as 98.5% and the agreement as 72.9% [30]. Thus, they concluded that sFLC measurements are less sensitive than sIFE, but more specific for detecting monoclonal proteins in serum. We also reached a similar conclusion in our study. In one study, abnormal serum FLC rates were found in 82% of all patients, including MGUS and SMM patients. In the remaining 18% patient group, MG could not be detected by sFLC [31].

In a similar study, sFLC values were found to be normal in only 50% of patients with MG detected by sIFE. Singhal et al. also found sFLC rates to be normal in 34% of patients with positive sIFE results [32]. While Katzmann et al. found 8% sensitivity of sPE and sFLC measurements alone without sIFE on MGUS scanning, they found an abnormal sFLC rate in only 42.4% of MGUS patients [13]. Again, in a few similar studies, inconsistencies were found between the 2 methods [28, 33]. All of these studies show us that sIFE or sFLC alone is not sufficient to detect MG.

In our study, we found that the sensitivity in detecting MG was 100% when we applied both sIFE and sFLC with sPE to all patients. In other words, when we used sIFE and sFLC simultaneously, we were able to detect all patients with BMb Clonal plasma cell ratios.

It is generally accepted that sPE/sIFE and urinary protein electrophoresis (uPE)/ urinary immunofixation electrophoresis (uIFE) should be evaluated together in adequate screening of MGs. One of the limitations of our study was the lack of 24-hour urine sampling due to logistical problems, loss of time, difficulties in patient cooperation and increased workload.

However, as a result of our study, we were able to detect all MGs with the simultaneous study of sPE, sIFE and sFLC, and showed that the need for a separate urine sampling could be eliminated.

Dispenzieri et al. [16] also found a result in parallel with our study, showing that the combination of sFLC, sPE and IFE is highly sensitive in screening myeloma and related diseases, and 24-hour urine studies are not needed. Sabatino [34] conducted a similar study to our study and showed that the combination of sPE ,sIFE and sFLC are sensitive and simple diagnostic tests for detecting MG . In our study, we tried to question the necessity of performing not only urine tests but also BMb. And indeed, we found that we could detect MG with sPE, sIFE, and sFLC in all patients diagnosed with BMb. In a similar study, Sidiqi et al. [35] showed that it is possible to diagnose with these methods, especially when diagnosing MGUS, without performing BM biopsies.

In other studies, in MG patients, there are studies showing inconsistency between IFE and FLC results [28, 33]. There could be many reasons for this inconsistency. One of these is usually a polyclonal increase in globulins secondary to inflammatory responses, particularly chronic inflammation and chronic liver disease, with an increase in κ light chain usually predominant. This increase can cause an abnormality in the sFLC value, which can lead to false negatives or false positives for MGs [26, 36-39]. In the study in which sPE and sIFE were used together, these two tests detected more than 90% of monoclonal Ig [36]. These methods were able to identify only 50% of intact monoclonal Ig and monoclonal light chain production, and intact Igs were detected in approximately 80% of MG patients. The remaining 20% of cases or light chain monoclonal proteins and nonsecretory lesions were missed by sPE and sIFE methods.

Another reason for false negativity can be explained by the inability to detect the group with free heavy chain disease by the sFLC method. In addition, impaired renal function also causes an increase in serum levels of free Ig light chains. Glomerular filtration depends on the size of proteins, and variability in polymerization of light chains also affects retention of free light chains differently. Normally, sFLC κ/λ is between 0.26-1.65, while this ratio is considered to be between 0.37-3.17 in patients with renal dysfunction due to these reasons [40-42].

For these reasons, sFLC cannot detect all of the intact Ig-induced gammopathies and light chain gammopathies, and its normal finding does not mean that there is no gammopathy [32, 43].

Therefore, abnormal sFLC value alone will not be sufficient to detect MGs.

Despite all these studies, it has also been shown that abnormal sFLC rates can be a good predictor of progression from SMM to MM [44].

Consequently, a test panel is required for screening MGs, as no single test has optimal sensitivity. In serum analysis, IMWG recommends a panel of sPE, siFE, and sFLC κ and λ tests for screening for pathological monoclonal proliferative disorders.

In our study, we found that 61.4% of 44 patients diagnosed with MG were male and 38.6% were female. In a study [5] conducted with 1384 patients, 54% of the patients were male and 46% were female. The study of Bin Xu et al. [45] supported this study and again found more male patients than females.

Clinical manifestations such as CRAB symptoms are among the diagnostic criteria of MM and indicate end-organ damage. MGUS is asymptomatic and has no CRAB findings. In our study, we evaluated patients for CRAB symptoms, and there was a significant difference between those with MG and those with PG in terms of hypercalcemia and osteoporosis. This situation can be explained by the presence of not only MGUS but also SMM and MM patients among the patients diagnosed with MG. In addition, the presence of these symptoms may predict the progression of MGUS to MM and may indicate the need for closer follow-up and even treatment of these patients compared to others.

In our study, we showed that in order to diagnose MGUS in patients with risk factors for MG, instead of looking at sIFE or sFLC alone, sFLC with sIFE can be as sensitive as BMb. Thus, we concluded that simultaneous monitoring of sIFE and sFLC when diagnosing MGUS may eliminate the need for BMb. However, prospective studies with a much larger number of patients are needed for this to be put into practical clinical practice

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