INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2025.93898
Int J Med Biochem 2025;8(3):185–191

Research Article

The importance of capillary protein electrophoresis in the early diagnosis and follow-up of monoclonal gammopathies

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Abstract

Objectives: Serum protein electrophoresis is a low-cost and low-sensitivity technique used for screening monoclonal gammopathies with capillary protein electrophoresis. Immunofixation electrophoresis, on the other hand, is a highly sensitive technique used in the diagnosis, treatment, and follow-up of monoclonal gammopathies in patients with peak or abnormal patterns in serum protein electrophoresis scans; however, it is also more expensive. In monoclonal gammopathies, excessive production of immunoglobulins, known as monoclonal bands, is observed as sharp bands in immunofixation electrophoresis. In this study, we aimed to highlight the contribution of serum protein electrophoresis and immunofixation electrophoresis in detecting monoclonal bands in laboratory reports and their role in the early diagnosis and follow-up of patients.

Methods: In this study, 781 serum protein electrophoresis and 144 immunofixation electrophoresis analysis reports were retrospectively evaluated. Of the 52 patients with deterioration in their SPE, 10 patients with SUD detected in their IFE were included in the study. The serum samples of the patients were analyzed using the Minicap Flex Piercing and Hydrasys 2 Scan Focusing analysis devices (Sebia, France).

Results: Among serum protein electrophoresis patterns, pathology (peak or distortion) was detected in 196 cases. Of these, peak was detected in 144 cases in SPE, while distortion was observed in 52 cases. For the evaluation of the disturbed electrophoretic patterns, immunofixation electrophoresis was recommended in the reports. Following clinical evaluation by the treating physician, immunofixation electrophoresis was performed for 26 patients. Monoclonal band was observed in 10 (38.5%) of these patients who underwent IFE study.

Conclusion: In our retrospective study, it was found that the frequency of monoclonal bands was high in patients with abnormal serum protein electrophoresis patterns where immunofixation electrophoresis was recommended. This underscores the importance of serum protein electrophoresis as a screening tool in the early diagnosis and follow-up of monoclonal gammopathies.

Keywords: Electrophoresis, monoclonal gammopathy, serum protein electrophoresis

How to cite this article: Dogan N, Gumus A, Ayer M. The importance of capillary protein electrophoresis in the early diagnosis and follow-up of monoclonal gammopathies. Int J Med Biochem 2025;8(3):185–191.

Immunoglobulin (Ig) is a glycoprotein composed of two identical heavy chains (α, γ, μ, δ, or ε) and two identical light chains (κ or λ), with a molecular weight of 55 kDa for the heavy chains (composed of 440 amino acids) and 23 kDa for the light chains (composed of 220 amino acids). The polypeptide chains are held together by covalent and non-covalent bonds, stabilized by disulfide linkages. There are five different heavy chain isotypes (IgG, IgA, IgM, IgD, and IgE) and two different light chain isotypes (κ and λ) [1]. Approximately 60% of immunoglobulins are of the κ chain type, and 40% are of the λ chain type. There is no functional difference between the κ and λ chains. In the initial response to an antigen, B lymphocytes secrete IgM and IgD. These lymphocytes later differentiate into plasma cells, which secrete larger amounts of immunoglobulins, mainly IgG, and also IgA and IgE, in response to the second dose of the same antigen [2].

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Submitted: November 12, 2024 **Revised:** March 25, 2025 **Accepted:** March 30, 2025 **Available Online:** June 17, 2025

Monoclonal gammopathy (MG) is associated with the clonal proliferation of plasma cells or B lymphocytes due to genetic or environmental factors. MGs are frequently observed in clinical conditions such as multiple myeloma (MM), plasmacytoma, plasma cell leukemia, Waldenström macroglobulinemia (WM), light chain amyloidosis, light chain deposition diseases, primary Amyloidosis (AL amyloidosis), POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes), and smoldering myeloma (asymptomatic multiple myeloma, SMM), as well as in MG of undetermined significance (MGUS) with low tumor burden, whose significance has not yet been established [3]. The measurement of monoclonal immunoglobulins is important for diagnosis, treatment, and monitoring. Laboratory techniques used in the screening and identification of MG have advantages and disadvantages in terms of specificity, sensitivity, and cost [2]. These laboratory techniques include serum and urine protein electrophoresis (SPE and UPE), serum immunofixation electrophoresis (IFE) and urine immunofixation electrophoresis (UIFE), nephelometric measurement of immunoglobulin heavy chains in serum, immunochemical measurement of free immunoglobulin light chain (κ and λ) components (nephelometric and turbidimetric), quantitative measurement of free κ and λ light chains, and the serum free κ/λ ratio. SPE is used as the initial screening test [4, 5].

Electrophoresis is a method in which proteins are separated based on their physical properties. For this, cellulose acetate, agarose, or fine capillaries are used as support media. The separation occurs according to the net charge, size, and shape of the protein when an electric current is applied. Cations from the buffer create an osmotic flow toward the cathode [6]. In non-gel techniques (such as capillary electrophoresis), there is no staining or washing process. Capillary electrophoresis is widely used because it provides rapid results and high-resolution separation. Other advantages of this technique are small sample volume and a wide range of detection methods. Other advantages are high selectivity, automation, linearity, reproducibility and use with mass spectrometry. The protein fraction is measured by optical densitometry, and an electrophoretic pattern is observed [7]. SPE consists of proteins from the albumin and globulin groups. Albumin is the largest peak and is located closest to the positive electrode. The proteins in the globulin group are divided into α1, α2, β1, β2, and γ globulins. The subgroups and relative amounts of these proteins are the primary focus in the interpretation of the electrophoretic pattern. In MG, the M-protein typically appears as a long, narrow, sharp peak in the α, β, or γ globulin regions of the electrophoretic pattern. If there is a polyclonal increase in immunoglobulins, a broad band is seen in the gamma region. In hypogammaglobulinemia, a reduction and flattening of the gamma globulin region is observed. Patients whose SPE shows no visible peaks but exhibit disturbances (such as decreases or flattening) in the bands should be monitored with follow-up SPEs to track their clinical course [8].

The evaluation of peaks in SPE, the detection of smaller M-proteins, and the identification of the heavy and light chain subtypes of M-proteins are more sensitive and costly methods, such as IFE. In IFE, the patient's serum is incubated with monospecific antibodies against the Ig heavy and light chains, and the resulting antigen-antibody complexes are assessed. Initially, antibodies against IgG, A, M, κ and λ are used. If no reaction occurs, the procedure continues with antibodies against IgD and IgE [5, 8].

Sharp MBs are observed in SPE, which is a screening test due to excessive production of immunoglobulins in MG. In our study, when IFE, which has high cost and sensitivity, is performed as a reflex test in patients with deterioration in beta and gamma regions in SPE, MB can be reported at a high rate. In the light of the data we obtained, we aimed to emphasize the importance of cost-effective use of laboratory tests in the early diagnosis and follow-up of MG in SPE deterioration.

Materials and Methods

The study included adult patients aged 18 and over who applied to the internal medicine and its subspecialties clinics of our hospital between 01.07.2022 and 31.10.2022, and for whom SPE and IFE tests were requested as part of their laboratory investigations. Of the 52 patients with deterioration in their SPE, 10 patients with SUD detected in their IFE were included in the study. Approval was received from the ethics committee of Basaksehir Cam and Sakura Hospital dated 25.01.2023, with protocol code 2023-36. Data were evaluated retrospectively in accordance with the Declaration of Helsinki. In our study, the data of the patients evaluated retrospectively were extracted from LIS (Laboratory Information System). During the study period, if multiple SPE and IFE tests were performed on the same patient, only the most recent sample was considered. Exclusion criteria was: Individuals aged 0–17 (not adults), patients who underwent SPE and IFE tests in units other than internal medicine and its subspecialties, and samples deemed unsuitable for pre-advantagetical evaluation (e.g., hemolyzed, lipemic, or icteric samples). In our study, an IFE test was not added as a reflex test. Among the patients with peak and/or deterioration in SPE, the doctor requested an IFE test for those with clinical indication.

SPE was performed using the Sebia Minicap (Sebia, Issy-les-Moulineaux, France) capillary zone electrophoresis kit. In the cases where pathology was not observed in the electrophoretic pattern of the six fractions constituting the total protein, peaks or peak(s) in the beta 1, beta 2, and gamma globulin fractions, where disturbances were observed, were marked manually, and explanations were added to the reports. For those patients whose SPE reports included additional explanations, IFE was performed using the Sebia Hydrasis 2 (Sebia, Issy-les-Moulineaux, France) agarose gel electrophoresis kit.

Statistical analysis

Data obtained from the study were classified based on parameters such as age, gender, date of application, referring unit, clinical diagnosis, and electrophoresis findings.

Table 1. Distribution of SPEs according to gender, age, clinical unit and diagnosis

	Those with distortions in the explanations of SPEs		IFEs requested from those with impaired SPE		Those with MCB detected IFE of those with in SPE disorder	
	n	%	n	%	n	%
Sex						
Female	34	65.3	14	53.8	4	40
Male	18	34.6	12	46.2	6	60
Age						
30–39 years	3	5.77	2	7.69	2	20
40–49 years	8	15.4	4	15.4	1	10
50–59 years	19	36.5	7	26.9	2	20
60–69 years	8	15.4	4	15.4	1	10
70–79 years	12	23.1	7	26.9	3	30
80 years old and above	2	3.85	2	7.69	1	10
Clinical unit						
Internal Medicine	9	17.3	3	11.5	2	20
Gastroenterology	1	1.92	–	–	–	–
Hematology	19	36.5	14	53.8	7	70
Nephrology	4	7.69	2	7.69	–	–
Rheumatology	19	36.5	7	26.9	1	10
Diagnosis						
Hematological disorders	6	11.5	4	15.4	2	20
Joint pain	18	34.6	8	30.8	2	20
Kidney diseases	6	11.5	3	11.5	–	–
General examination	12	23.1	3	11.5	–	–
Monoclonal gammopathy	3	5.77	2	7.69	2	20
Multiple Myeloma	7	13.4	6	23.1	4	40

SPE: Serum protein electrophoresis; IFE: Immunofixation electrophoresis; MCB: Monoclonalband.

Results

The distribution of the patients with disturbances in the explanations of the SPEs, the IFE tests performed on those with disturbances, and the IFE samples showing MCBs according to gender, age, clinical unit, and diagnosis are shown in Table 1.

The evaluation of the cases in our study is given in Figure 1.

A total of 781 SPE and 144 IFE tests were included in the study. Of the analyzed SPE tests, 74.90% (n=585) showed no pathology, while 25.10% (n=196) showed pathology. Among the SPE tests evaluated as pathological in the study, 18.43% (n=144) showed a manually marked peak in the electrophoresis pattern, and 6.55% (n=52) exhibited a disturbance in the six fractions of total protein.

In the study, 144 SPE tests with manually marked peaks were analyzed using IFE in 64.58% (n=93) of the cases. Monoclonal bands (MCB) were observed in 66.66% (n=52) of the IFE samples tested from the patients with marked peaks in the SPE report. 52 SPE tests showing disturbances in the six fractions of total protein were analyzed with IFE in 50% (n=26) of the cases. MCB were observed in 38.5% (n=10) of the IFE samples tested from the patients who showed disturbances in the six fractions of total protein. In our study, the electrophoretic pattern of the control sample in the SPE was evaluated as normal (Fig. 2). In Figure 2, a narrow and sharp peak is observed in the first albumin band. The alpha 2 (α2) globulin band is wider and higher than the alpha 1 (α1) globulin band; the beta 1 (ß1) globulin band is higher and sharper than the beta 2 (ß2) globulin band; and the gamma globulin band has an inverted "T" shape.

In the electrophoretic patterns of the patients in our study, disturbances were reported in the beta 2 and/or gamma globulin bands. In these disturbances, the beta 2 globulin band is higher and sharper than the beta 1 globulin band, with visual defects such as depressions and peaks on the surface of the beta 2 globulin band and gamma globulin band. To visually evaluate these disturbances, the relevant bands were zoomed in on using the device. If the disturbances become clearer upon zooming, it was recommended to perform IFE for the evaluation of the disturbances in the relevant bands and/or peaks. In our study, MCB were detected by IFE in patients with disturbances in the beta 2 and/or gamma globulin bands (Fig. 3). MCBs found in the IFE of cases with pathological disturbances in the electrophoretic pattern of the SPE are shown in Figure 4.

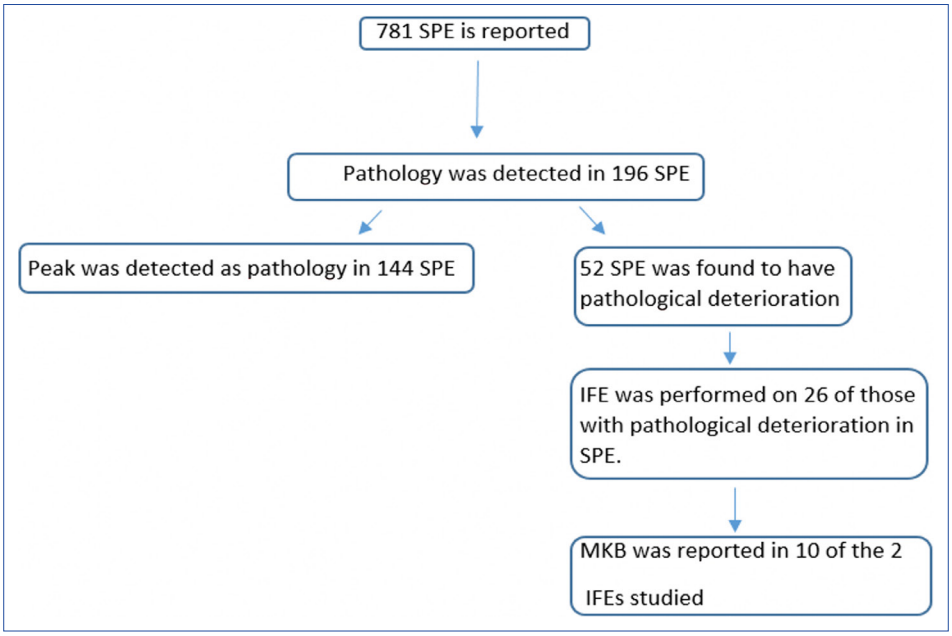

Figure 1. Evaluation scheme of the cases in our study.
SPE: Serum protein electrophoresis; IFE: Immunofixation electrophoresis; MCB: Monoclonalband.

Discussion

MG is a group of diseases characterized by the proliferation of one or more clones of differentiated plasma cells. It is characterized by the detection of MCB in the patient's serum and urine [8]. The frequency of MG in plasma cell dyscrasias is observed in 7% of all hematologic malignancies. MG observed in plasma cell dyscrasias is generally associated with MM, amyloidosis, Waldenström's macroglobulinemia (WM), or plasmacytoma. MG cases without such associations are termed as MG of undetermined significance (MGUS) [9, 10]. In our study, 38.5% (n=10) of the patients with pathological changes in the SPE had MCBs detected by IFE. In patients suspected of having MG, the lower-cost SPE was used as a screening test to evaluate any pathologies in the electrophoretic pattern. As a result of these evaluations, patients with abnormalities in the SPE were then tested with the more expensive IFE, which showed a higher frequency of MCB detection. Literature shows that the prevalence of MG is associated with increasing age and male gender. In some studies, MM is slightly more common in women than in men [11]. However, MGUS is observed at least 1.5 times more frequently in men than in women [12]. Since SPE is more commonly requested as a screening test in women, pathological findings (peaks and/or disturbances) are expected to be more frequently reported in women. In our study, disturbances in the SPE were more commonly observed in women, while MCBs detected by IFE were more frequent in men.

MG can develop at any age, but its frequency increases with age. The frequency of MCB occurrence is 1.7% in individuals over 50 years old and 3.6% in individuals over 70 years old [13]. When these findings were divided into 10-year age groups for those over 50, the frequency of MG was found to be 3.91%, 5.72%, 7.75%, 8.67%, and 12.75% for those over 90 years old [14]. MM

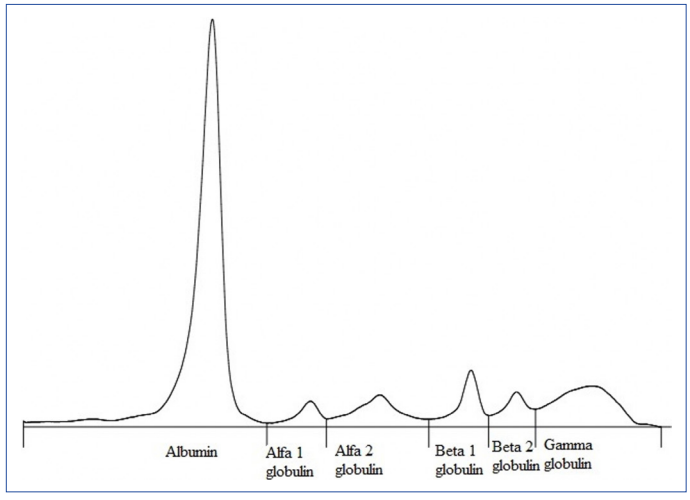

Figure 2. Visualization of normal electrophoretic pattern in control sample in SPE.

is the most common form of MG, and the age of diagnosis is typically between 66 and 70 years. MM has an asymptomatic premalignant stage referred to as MGUS, and it is observed that 1% of MGUS patients progress to MM each year. The frequency of MGUS is 1.7% in individuals over 25 years of age, 3% in those over 60, and 10% in individuals over 80 years old [15]. In our study, the age distribution of patients with disturbances in the SPE and the age distribution of patients with MCBs detected by IFE were found to be compatible. The frequency of MCB detection by IFE was higher in patients over 40 years old with disturbances in the SPE, indicating that early diagnosis and treatment of MG were effectively facilitated. Studies have shown that changes in the gamma band of the SPE pattern are observed in polyclonal gammopathy (PG), MG, and hypogam-

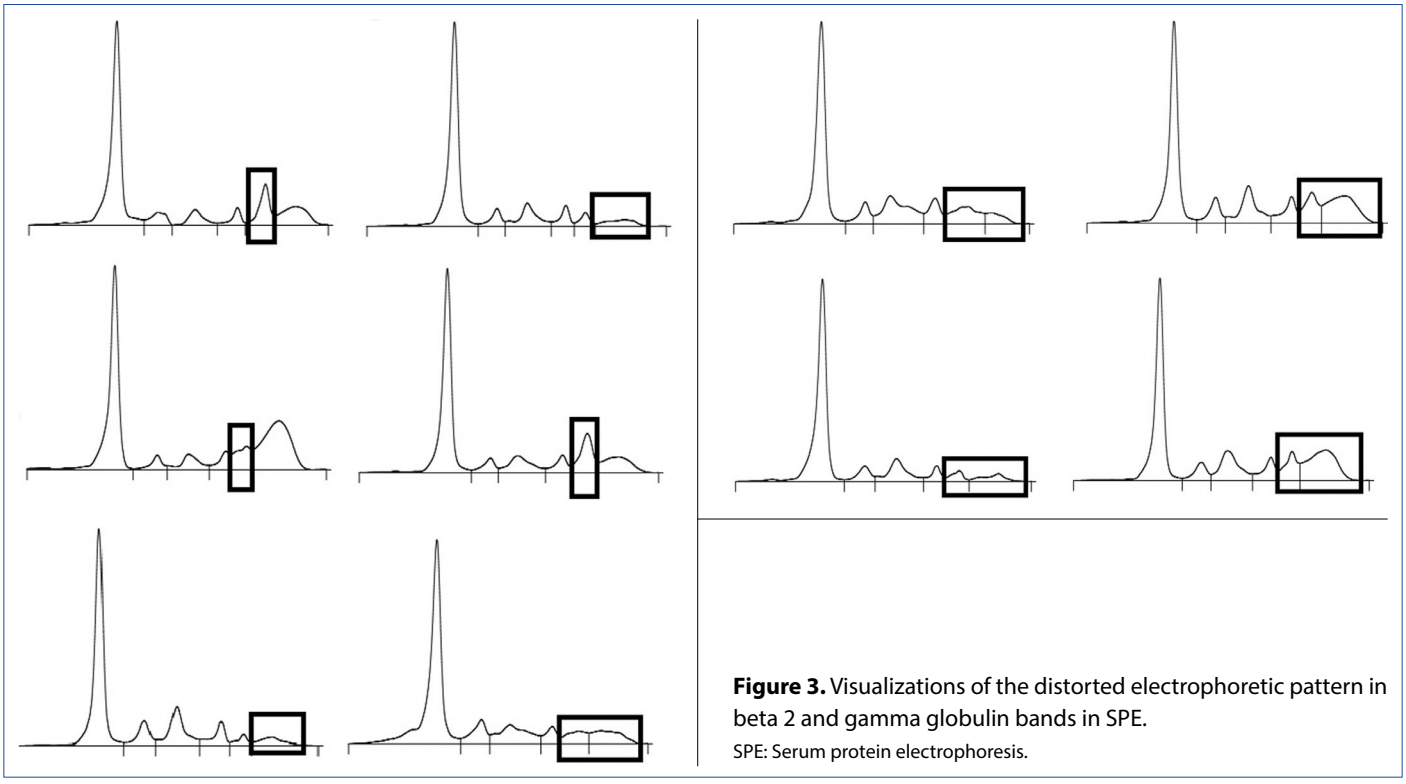

Figure 3. Visualizations of the distorted electrophoretic pattern in beta 2 and gamma globulin bands in SPE.
SPE: Serum protein electrophoresis.

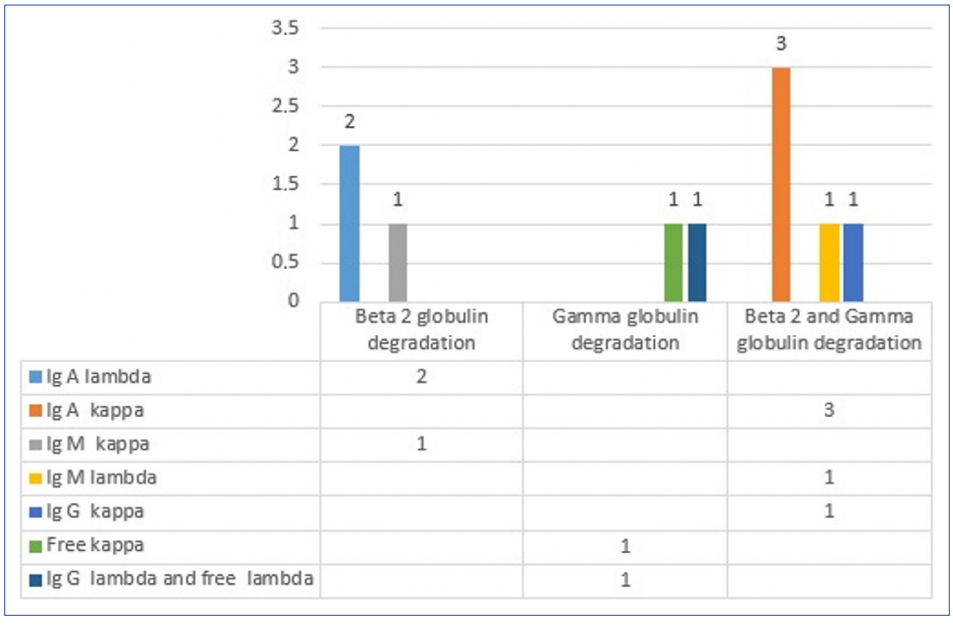

Figure 4. Distrubution of MCB's observed with IFE in patients with deterioration in SPE.
MCB: Monoclonalband; SPE: Serum protein electrophoresis.

maglobulinemia. Of PG patients, 61% have liver disease, 28% have connective tissue diseases, and 8% have chronic infections. Hypogammaglobulinemia has also been observed due to hereditary reasons or during the course of malignant or benign diseases. In the literature, MGUS was observed in 51% of MG patients, MM in 18%, AL amyloidosis in 11%, lymphoproliferative diseases in 4%, smoldering multiple myeloma (SMM) in 6%, WM in 3%, and solitary or extramedullary plasmacytomas in 1% of cases [16, 17]. In our study, among the patients with disturbances in the SPE (n=52), 36.5 % were from hematology, 36.5 % from rheumatology, 17.3% from internal medicine, and 7.69% from nephrology. Among those with disturbances in the SPE, 70% of the IFE tests that detected MCBs were from hematology, 20% were from internal medicine, and 10% were from rheumatology. The higher frequency of MCB detection in hematology was due to the greater number of patients diagnosed and fol-

lowed for MM, MG, and other paraproteinemias. In the internal medicine department, IFE was requested for patients suspected of MG, and the frequency of MCB detection was higher. In rheumatology, although SPE was frequently requested as part of the screening process, MCB detection was lower due to the lower frequency of MG and other paraproteinemias.

Diseases with paraprotein-associated kidney involvement require a collaborative approach between hematology and nephrology. Cast nephropathy is the most common renal lesion seen in MM and is one of the most important causes of kidney failure. MG of renal significance (MGRS) is used in nephrology practice to describe cases where monoclonal paraprotein causes renal damage without meeting the criteria for hematologic malignancy. MGRS is defined as a clonal proliferative disorder that does not meet the criteria for hematologic malignancy but produces nephrotoxic monoclonal immunoglobulins. MGRS encompasses all B-cell lymphoproliferative and plasma cell proliferative disorders that do not meet the criteria for MM, WM, chronic lymphocytic leukemia (CLL), or Malignant Lymphoma. Kidney lesions associated with monoclonal Ig in low-grade CLL and low-grade B-cell non-Hodgkin lymphoma are also included in MGRS. Additionally, if kidney involvement is present in MM, primary AL amyloidosis, or MGUS, it is also considered MGRS [18]. In MGRS, kidney damage can be glomerular, interstitial, mesangial, or tubular. Without treatment to suppress the clonal production, MGRS can progress to end-stage kidney failure. In our study, among the two patients with disturbances in the SPE from nephrology, no MCBs were detected in their IFE results. For patients suspected of MG and/or MGRS, kidney biopsies should include immunofluorescent and immunohistochemical evaluations alongside light microscopy, and electron microscopy should also be considered for a more comprehensive assessment. In our study, of the patients with disturbances in the SPE (n=52), 34.62% had joint pain, 23.08% had general symptoms such as fatigue and vitamin deficiencies, 19.22% had MG and/or MM, 11.54% had hematologic symptoms, and 11.54% had kidney diseases. Among the patients with disturbances in the SPE who had MCBs detected by IFE (n=10), 60% were diagnosed with MG and/or MM, 20% had joint pain, and 20% had hematologic symptoms. Joint pain, fatigue, and vitamin deficiencies are common clinical symptoms observed in many hematologic malignancies, including MG and MM [3, 8]. These clinical symptoms are frequently seen in nonspecific clinical departments such as rheumatology, nephrology, neurology, and orthopedics. Since SPE is requested as part of the screening process in these settings, the detection rate of MCBs by IFE is lower. Since the diagnostic and monitoring criterion for MG and/or MM is the detection of MCBs in serum, IFE is more frequently used to detect MCBs in patients with disturbances in their SPE. In the literature, it has been shown that even when serum total protein, beta, or gamma globulin levels are within normal limits, a small MCB may be hidden in the beta or gamma regions [19, 20]. In MG screenings, when MCBs are hidden, measurements of immunoglobulins (IgG, IgA, IgM) and total light chains (kappa and lambda) by SPE are often insufficient. Therefore, IFE, which has higher sensitivity than SPE, and quantitative measurements of free light chains in serum are recommended for diagnosing MG [20]. In patients with MCBs detected by IFE, IgG kappa was found in 35.3%, IgG lambda in 10.8%, and IgA kappa in 6.1% of cases [20–22]. In our study, among the patients with disturbances in the SPE, MCBs detected by IFE were found in 30% of cases as IgA kappa, 20% as IgA lambda, 10% as IgM kappa, 10% as IgM lambda, 10% as IgG kappa, 10% as free kappa, and 10% as two or more immunoglobulins with light chains (IgG lambda and free lambda). Capillary electrophoresis (CE) plays a critical role in the early detection and monitoring of monoclonal gammopathies due to its high resolution and automation capabilities. Its ability to efficiently separate protein fractions allows for the identification of subtle abnormalities that may indicate the presence of monoclonal proteins. Compared to traditional gel-based methods, CE offers faster turnaround times, reduced sample volume requirements, and improved reproducibility, making it highly valuable in routine clinical laboratories. The detection of electrophoretic disturbances, particularly in the beta and gamma regions, by CE enables the timely recommendation of confirmatory tests such as immunofixation electrophoresis. Therefore, incorporating capillary electrophoresis into diagnostic workflows significantly enhances the sensitivity of screening for monoclonal gammopathies and supports early clinical decision-making. The most important limitation of our study is that IFE, which is a costly test, cannot be performed on every patient for whom SPE is requested. In addition, since ethics committee approval was obtained for adult patients from internal medicine and its subspecialties, data from adult patients from other branches could not be included in the study. Samples that are preanalytically inappropriate in the laboratory (hemolysis, lipemia) are also not included in the study.

Conclusion

In our retrospective study, the frequency of MCBs was found to be high in patients with disturbances in their SPE who were subsequently tested with IFE. This highlights the importance of SPE in the early diagnosis and follow-up of MG, which can present in various clinical scenarios. The detection of MCBs in MG can be effectively screened with SPE, which is faster and more cost-effective compared to IFE. We recommend performing IFE as a reflex test in patients with abnormalities detected in their SPE electrophoretic pattern, as it significantly aids clinicians in making diagnostic and treatment decisions.

One of the main limitations of this study is the study population was restricted to adult patients from internal medicine and its subspecialties, limiting the generalizability of the findings to other clinical departments. Additionally, pediatric patients and samples with pre-analytical issues such as hemolysis, lipemia, or icterus were excluded, potentially reducing the sample diversity. The retrospective design of the study may also introduce selection bias, as only cases with available laboratory records were included. Finally, the lack of long-term clinical follow-up data prevents evaluating the progression of detected monoclonal bands to clinically significant disease.

Ethics Committee Approval: The study was approved by the Basaksehir Cam and Sakura Hospital Clinical Reseach Ethics Committee (no: 2023-36, date: 25/01/2023).

Informed Consent: Informed consent was obtained from all participants.

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

Funding: The authors declared that this study received no financial support.

Use of AI for Writing Assistance: No AI technologies utilized.

Authorship Contributions: Concept – N.D., A.G., M.A.; Design – N.D., A.G.; Supervision – N.D., A.G., M.A.; Funding – N.D., A.G., M.A.; Materials – N.D., A.G., M.A.; Data collection and/or processing – N.D., A.G.; Data analysis and/or interpretation – N.D., A.G.; Literature search – N.D., A.G., M.A.; Writing – N.D., A.G., M.A.; Critical review – A.G., M.A.

Peer-review: Externally peer-reviewed.

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