ISSN Print: 2664-6188
ISSN Online: 2664-6196
Impact Factor: RJIF 5.35
IJCBB 2023; 5(1): 47-51
www.biochemistryjournal.net
Received: 12-10-2022
Accepted: 16-11-2022

Adam Bentahila
Resident in Medical, Department of Biology Immuno-Hematology Laboratory Abulcassis, University of Health Sciences, (Uiass) Rabat, Morocco

Mohamed Wali Alami
Resident in Medical Biology, Department of Hematology Laboratory, Abulcassis University of Health Sciences (Uiass), Rabat, Morocco

Imane Hadi
Resident in Medical Biology, Department of Hematology Laboratory, Abulcassis University of Health Sciences (Uiass), Rabat, Morocco

Sami Zarhloul
Resident in Medical Biology, Department of Microbiology Laboratory, Abulcassis University of Health Sciences (Uiass), Rabat, Morocco

Abdelhamid Zrara
Head, Department of Immunology Laboratory, Faculty of medicine and pharmacy Mohamed V, Rabat, Morocco

Amina Benouda
Head, Department of the Cheikh Zaid Laboratory, Abulcassis University of Health Sciences (Uiass), Rabat, Morocco

Corresponding Author:
Adam Bentahila
Resident in Medical, Department of Biology Immuno-Hematology Laboratory Abulcassis, University of Health Sciences, (Uiass) Rabat, Morocco

Comparative study of serum free light chain assay and urine protein immunofixation in the diagnosis of patients with monoclonal gammopathy

Adam Bentahila, Mohamed Wali Alami, Imane Hadi, Sami Zarhloul, Abdelhamid Zrara and Amina Benouda

DOI: https://doi.org/10.33545/26646188.2023.v5.i1a.39

Abstract
Serum protein electrophoresis and urine protein immunofixation (IF) are biological techniques for the diagnosis and monitoring of hematological malignancies. The advent of serum free light chain (FLC) assay has provided a new diagnostic element for monoclonal gammopathies. The study conducted in the medical biology laboratory of the Cheikh Zaid University Hospital in Rabat between 2020 and 2022 showed that the determination of serum free light chains (FLCs) provides more critical and accurate information for both the diagnosis and monitoring of hematological malignancies such as light chain myeloma.

Keywords: Multiple myeloma, bence Jones protein, light chains, kappa lambda ratio, renal failure

Introduction
Multiple myeloma (MM) is a malignant tumor of plasma cells, which are involved in the production of monoclonal immunoglobulins. A characteristic feature of this plasma cell dyscrasia is the secretion of monoclonal immunoglobulins, often called monoclonal M proteins, which can be used as a diagnostic or monitoring marker for the disease. The classification and differential diagnosis of monoclonal gammopathies is based on clinical, biological, and radiological criteria but remains difficult in some cases. MM is the most common malignant gammopathy and is associated with a broad spectrum of clinical signs and symptoms [1]. The heavy and light chain components of the M protein can be identified by immunofixation and quantified by serum protein electrophoresis and/or by a serum free light chain (sFLC) assay [2]. Immunofixation is much more sensitive than electrophoresis and can identify the monoclonal M protein involved [3]. Myeloma evolves in 3 distinct phases: monoclonal gammopathy of undetermined significance (MGUS), smoldering myeloma (SMM) and MM, which are classified according to CRAB criteria and SLIM (Biomarker) markers [4, 5] (Figure 1).
Serum electrophoresis and immunofixation may not be able to detect light-chain abnormalities in patients with oligosecretory disease, such as light chain deposition disease (LCDD). Because of their low molecular weight, these sFLC are rapidly cleared by the kidneys. In this case, the monoclonal load should be measured in a 24-hour urine collection or in serum by automated FLC immunoassay. The latter has a higher sensitivity to detect and quantify the free light chains involved. In accordance with the International Myeloma Working Group (IMWG) for the diagnosis of a monoclonal plasma cell disorder, it is recommended to perform serum immunofixation and electrophoresis on serum and urine samples as well as FLC assay [1]. Other tests should also be performed in the laboratory as part of the diagnosis and follow-up of these patients: a blood count (to look for cytopenia), blood smear (to look for circulating plasma cells) and other biochemical tests that assess renal (β2-microglobulin) and hepatic function. Proteinuria should be assessed on urine samples from all patients at diagnosis and during follow-up. If proteinuria is present, it should be quantified in a 24-hour urine collection. Total 24-hour proteinuria and Bence-Jones proteinuria (BJP) (excretion of monoclonal free light chains in the urine of patients with gammopathy) should be assessed by densitometry, electrophoresis, and immunofixation. Quantification of BJP is important in defining the diagnosis of smoldering

MM (SMM) and is also important in assessing the response to treatment [6]. These 24-hour urine collections are often performed irregularly, resulting in incomplete urine collection. Renal function can also influence the accuracy of the results, which must be taken into account when interpreting the results [1]. The development of sFLC assay kits has challenged the IF of urine proteins. In patients with LCDD, kappa or lambda (κ or λ) light chains are produced in excess, resulting in an increased or decreased ratio of FLC κ to FLC λ (ratio κ/λ) [7]. Catabolism and reabsorption of light chains occur primarily in the proximal tubules of the kidney [8, 9], so that measurable FLC in urine represents overflow proteinuria and is under the influence of renal function status as well as tumor light chain production. This is a direct consequence of renal light chain metabolism and shows that the FLC assay is more sensitive than BJP assays for the detection of abnormal excess light chain production [10, 11]. Indeed, some studies have shown a higher sensitivity of FLC assay compared to urine protein IF in patients with IgG or IgA myeloma [12].

It is in this context that the present study will compare the sensitivity of BJP and the concentrations of sFLC in patients with MM, in order to evaluate whether the quantification of sFLC could be used as a substitute for urinary monoclonal protein measurements in patients with MM.

Materials and Methods

Patients

The study included 22 patients, most of whom had monoclonal gammopathies and were hospitalized at Cheikh Zaid University Hospital in Rabat between 2020 and 2022.

The clinical diagnosis of MM was made according to international diagnostic criteria [13, 14].

The following parameters were noted at the time of diagnosis: age, gender, creatinine, glomerular filtration rate calculated from serum creatinine using the CKD-EPI (Chronic Kidney Disease - Epidemiology Collaboration) study equation, β2-microglobulin, monoclonal protein isotype, urinary immunoglobulin/24 hours, and percentage of plasma cells in the bone marrow.

Method

Protein electrophoresis of serum and 24-hour urine, and immunofixation of serum proteins were performed on agarose gels (Hydragel 1 Bence Jones, Hydragel 4 Bence Jones, Hydragel 1 IF, Hydragel 4 IF).

The following antisera were used: anti-Hum. IgG, anti-Hum. IgA, anti-Hum. IgM, anti-Hum. Kappa, anti-Hum. Lambda, anti-Hum. Free light chains Kappa, anti-Hum. Lambda free light chains, anti-Hum. Ig G.A.M.

In each case, the presence of intact/fragmented immunoglobulin in the urine was confirmed by a specific positive antiserum reaction.

BJP analysis was performed on unconcentrated urine. The detection level of Bence Jones protein is generally within 1 - 5 mg/dL. It should be noted that the urinary protein level was not measured.

The FLC assay was performed on an immunoturbidimetric automaton, the SPAplus™ (The Binding Site, UK), following the manufacturer's instructions, with fresh or frozen sera.

The Freelite™ assay (The Binding Site, UK) is a turbidimetric assay that uses polyclonal antibodies directed against epitopes of the FLC constant region that are normally hidden in intact immunoglobulins; therefore, only light chains that are not bound to a heavy chain are quantified.

Serum is diluted 1:10 for SPAplus™ and allows quantification ranging from 4-180 mg/L and 4.5-165 mg/L for detection of FLC κ and λ respectively.

The ratio of the FLC κ/FLC λ assay was compared with normal reference values (ratio κ/λ ranging from 0.26-1.65) [15].

Results

The median age of the patients (13 men and 9 women) was 64 years. 3 patients were suffering only from LCDD λ and 1 patient was suffering from non-secretory myeloma with FLC κ. 11 patients had multiple myeloma (6 with IgG κ, 1 with IgG λ, and 4 without isotype determination), 1 patient had type κ biclonal gammopathy, 1 patient had AL amyloidosis, and 2 patients had no monoclonal gammopathy (Table 1).

14 patients had a positive BJP (10 free κ and 4 free λ) and of these 14 patients 100% had an abnormal κ/λ ratio. 3 patients had no BJP but had an abnormal κ/λ ratio.

Bence Jones proteinuria was detected in all patients with LCDD, non-secretory multiple myeloma and in the patient with AL amyloidosis.

In contrast, BJP was detected in only 50% of patients with IgG κ multiple myeloma and was not detected in the patient with IgG λ myeloma (Table 2).

Creatinine and GFR (Glomerular filtration rate) calculations were reported for 17 patients. Of the 17 patients, 5 patients had a GFR < 15, 3 patients had a GFR between 15 and 29, 3 had a GFR between 30 and 59 and 6 patients had a GFR greater than 60.

According to the French National Authority for Health, 8 patients therefore had severe or even end-stage chronic renal failure.

The 11 patients with a GFR lower than 60 had an abnormal ratio κ/λ and positive Bence Jones proteinuria (Table 3).

Myelograms were performed in 13 patients. Four patients had a plasma cell count of less than 10% and nine patients had a plasma cell count of more than 10%, including one patient with a count of 90% (Table 4).

Of the patients with a level below 10%, 3 patients had no BJP and of these 3 patients, only one had a normal ratio.

All patients with a plasma cell count above 10% had a positive BJP and an abnormal κ/λ ratio.

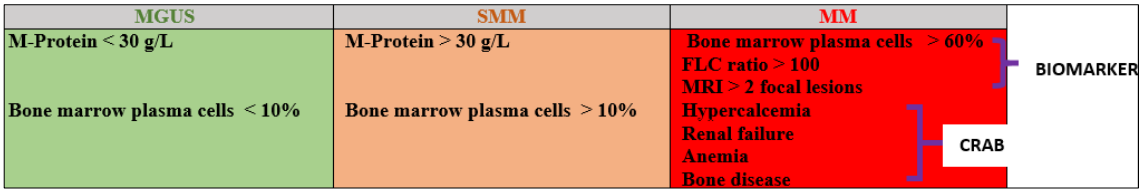

Fig 1: Difference between MGUS, SMM and MM

Table 1: Table 1. Characteristics and biological results of patients (n = 22).

	n	Mean	Median (Range)
Age (Years)		64	63 (52-80)*
Creatinine mg/L		36.4	24.5 (5.76-111)**
Bence Jones protein positive	14		
Free κ	10		
Free λ	4		
Bence Jones protein negative	8		
Serum free light chain dosage			
κ mg/L	22	503.27	68.30 (7.91-4800)
λ mg/L	22	127.45	18.06 (0.86–1559)
Normal ratio κ/λ***	5		
Abnormal ratio κ/λ	17		

n: Number of patients
* Data missing for three patients
** Data missing for five patients
*** Normal ratio κ/λ according to IMWG: 0.26 < / < 1.65

Table 2: Outcome of Bence Jones proteinuria and serum light chain assay ratio κ/λ according to pathology.

	Bence Jones proteinuria (positive)	Bence Jones proteinuria (Negative)	Abnormal ratio κ/λ	Normal ratio κ/λ
Light chain deposition disease (n = 3)	3	0	3	0
Non-secretory multiple myeloma (n = 1)	0	1	1	0
IgG κ multiple myeloma (n = 6)	3	3	5	1
IgG λ multiple myeloma (n = 1)	0	1	1	0
Biclonal gammopathy type κ (n = 1)	1	0	1	0
Multiple myeloma without iso type determination (n = 4)	4	0	4	0
AL Amyloidosis (n = 1)	1	0	1	0
Absence of monoclonal gammopathy (n = 2)	0	2	0	2
Patient without diagnosis (n = 3)	2	1	2	1

Table 3: Assessment of renal activity based on Bence Jones proteinuria and free light chain assay ratio κ/λ (n = 17).

	Mean creatinine level (mg/L) and normal ratio κ/λ (0.26 < κ/λ < 1.65) (n = 1)	Average GFR (mL/min/1.73m2) And normal ratio κ/λ (0.26 < κ/λ < 1.65) (n = 1)	Mean creatinine level (mg/L) and abnormal ratio κ/λ (κ/λ > 1.65 κ/λ < 0.26) (n = 16)	Average GFR (mL/min/1.73m2) and abnormal ratio κ/λ (κ/λ > 1.65 κ/λ < 0.26) (n = 16)
Bence Jones proteinuria (Positive)	AP*	AP	44.64 (n = 13)	37
Bence Jones proteinuria (Negative)	8.46	98	9.79 (n = 3)	95

Table 4: Classification of patients who underwent myelograms according to the ratio κ/λ and Bence Jones proteinuria (n = 13).

	Normal ratio κ/λ BJP (Negative)	Abnormal ratio κ/λ BJP (Negative)	Abnormal ratio κ/λ BJP (Positive)
Bone marrow plasma cells < 10%	1	2	1
Bone marrow plasma cells > 10%	0	0	9

Table 5: Comparison of sFLC assay results with the results of investigation in urine.

	N	Mean (range) of serum FLCκ values (mg/L)	Mean (range) of serum FLCλ values (mg/L)	Mean (range) of κ/λ ratio	Number of patients with abnormal κ/λ ratio	Number of patients with normal κ/λ ratio
Protéinurie de BJ (positive)	14	756.38 (10.48- 4800)	179.82 (0.86-1559.19)	153.32 (0.008-321.74)	14	0
Protéinurie de BJ (négative)	8	60,34 (7.91-302.07)	20,70 (4.52-69.67)	6.39	3	5

Discussion

Of the 22 patients in the study, 17 were diagnosed with monoclonal gammopathy. Of these 17 patients, 94% had an abnormal κ/λ ratio and 70% had a positive PBJ. Thus, the PBJ was negative in 30% of the patients while only 6% of the patients with monoclonal gammopathy had a normal κ/λ ratio. For the latter, it was a patient with IgG myeloma and the ratio was 1.62.

These results show that the FLC assay provides more accurate diagnostic information than BJP (Table 5).

This study shows that the FLC assay remains a more sensitive and earlier marker than the urine protein IF. Indeed, in the literature, several studies conducted in this direction have reported the same conclusion. Studies have shown the excellent sensitivity of the FLC assay for the detection of FLC in patients with LCDD [12]. Urine protein electrophoresis is still recommended for monitoring the response to treatment in patients with LCDD not measurable by serum protein electrophoresis [16]. It should be mentioned that some authors express somewhat different opinions.

Siegel *et al*. [17] reported that FLC analysis provides a more accurate measure of light chain burden than urine protein electrophoresis. It has been proposed that the combination of serum electrophoresis and FLC assay evaluation be incorporated into an algorithm for the evaluation of monoclonal gammopathies [17, 18].

Studies have shown that serum normalization of FLC concentration correlates with clinical efficacy of treatment [19, 20]. Other studies show that urine test results underestimate the amount of sFLC produced and overestimate the response to therapy due to renal reabsorption and metabolism of sFLC [21].

In general, myeloma patients with renal involvement have serum FLC concentrations above 500 mg/L [22, 23] and a threshold of 500 mg/L (according to the updated IMWG criteria > 1500 mg/L [4]) is often considered a level above which nephropathy is a likely pathology.

A study of 122 myeloma patients conducted by the Medical Research Council in the United Kingdom showed that normalization of serum free light chain concentrations as well as normalization of the κ/λ ratio represented prognostic factors for improved overall survival [24].

A French study demonstrated the rapid disappearance of FLC in urine in a disproportionate percentage of patients after only 2 cycles of treatment, compared with serum assessment. This suggests that quantification of sFLC may better reflect tumor response to treatment than testing for BJP [25].

The superior sensitivity of sFLC compared with urinary measurements provides additional clinical information, justifying the use of serum rather than urine assessments for follow-up of patients with MM [25].

The sensitivity of the tests is likely to be influenced by renal function [10, 26]. A previous study suggested that serum concentrations of 133 mg/L kappa light chains and 278 mg/L lambda light chains are required to exceed the reabsorptive capacity of the kidney and allow detection of FLC in urine [10]. The impact of load [27] and blood pressure [28] can influence the presence of FLC in urine, and make these thresholds somewhat subjective. The assessment of sFLC can also be affected by renal function, with a proportional increase in kappa FLC concentrations relative to lambda FLC concentrations as renal function deteriorates, which can lead to a shift in the FLC κ/λ ratio. Therefore, a renal reference range has been proposed that corrects for renal function in patients with nephropathy [25].

However, it has been shown that there is not a strong correlation between serum FLC concentration and 24-hour urine measurement [16, 26]. The replacement of 24-hour urine testing with FLC testing for all myeloma patients remains controversial. A study by the Eastern Cooperative Oncology Group of 399 patients with MM (only a minority of whom had LCDD) found only a weak correlation between sFLC test results and 24-h protein analysis [29]. For patients with measurable urinary M proteins, MM should be monitored by 24-h urine collections. When albumin is the dominant protein found in the urine, glomerular nephropathy (such as AL amyloidosis) should be excluded [1]. The 24-hour urine collection remains important when results are discordant. Early detection of PBJ in urine may improve the patient's prognosis, as the level of this protein is directly related to renal function as well as the rate of its deposition in tissues [6].

Ultimately, several studies [12, 21] have demonstrated that the FLC assay is a more sensitive marker for diagnosis and monitoring of disease treatment than urine analysis. This assay provides more accurate prognostic information, demonstrating the greater relevance of measuring these light chains [21]. Nevertheless, the IMWG still recommends the measurement of BJP [3, 13, 30] for the monitoring and evaluation of the response to treatment especially in patients with SMM, LCDD and AL amyloidosis.

Conclusion

The comparative study conducted on this sample shows more significant results obtained by the FLC assay than those obtained by the BJP assay. Several papers showed similarities in the final results.

BJP remains a marker of choice for SMM and for the evaluation of renal involvement. New urinary immunofixation tests have been developed that allow, in addition to the detection of BJP, the detection of other proteins of nephrological interest. These tests have the advantage of not requiring a concentration of urine and can therefore avoid the biases associated with the analytical and pre-analytical phase. These new innovations will provide the clinician with information on potential kidney damage with additional in-depth analysis in order to prevent renal failure and start early therapy.

In conclusion, even if it has been demonstrated the higher sensitivity of the FLC assay in the diagnosis of monoclonal gammopathies, BJP still remains a marker that has its importance in these pathologies.

Acknowledgments

I thank my department head, the hospital director, and the ethics committee for allowing me to conduct this study.

I declare that I have no conflicts of interest.

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