Serum and Urine Free Light Chain Cut-off Detection for a Rational Employment of the Bence-Jones Proteinuria Test in Presence of Monoclonal Gammopathy

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ABSTRACT

We evaluated whether, in presence of monoclonal gammapathy (MG), serum and urinary free light chains (FLCs) measurement may limit the Bence-Jones proteinuria (BJP) test employement, that is affected by pre-analytical, analytic and post-analytical problems. From March to May 2016, 89 patients who were refered to our laboratory for BJP assay with suspected plasma cell dyscrasia, were evaluated. In presence of MG, it was typified by serum immunofixation electrophoresis. Moreover, sFLC, uFLC and related FLC-κ/λ ratio, as well as glomerular filtration rate (eGFR), were also measured. Patients with MG-κ: the ROC curves analysis showed an accurate comparison between sFLC-κ and sFLC-κ/λ ratio vs BJP-κ positivity detection. The urinary data showed a good correlation between uFLC-κ vs BJP-κ positivity detection, whereas the correlation between uFLC-κ/λ ratio vs BJP-κ positivity detection did not show satisfactory results. Patients with MG-λ: comparing sFLC-λ and sFLC-κ/λ ratio vs BJP-λ positivity detection, the ROC curves analysis showed a good correlation. The urinary data of uFLC-λ vs BJP-λ positivity detection and uFC-κ/λ ratio vs BJP-λ positivity detection did not show satisfactory results for sensitivity and specificity. The absolute values of "k" and "λ" FLC vs BJP positivity detection, statistically are more significant than the FLC-κ/λ ratio. The aggregate data of uFLC, [uFLC-Σ(κ+λ)], identified a statistically significant median value for the detection of BJP positivity. Although the study statistically showed the possibility in same cases to optimize the BJP test employment, further validations to confirm the objectives by us defined are needed.

Keywords: Plasma cell dyscrasia, Monoclonal gammopathy, Serum free light chain, Urine free light chain, Bence-Jones proteinuria

ÖZET

Monoklonal Gammopati Varlığında Bence-Jones Proteinüri Testinin Akılcı Kullanımı İçin Serum ve İdrar Serbest Hafif Zincir Cut-off Tespiti

Monoklonal gammapatinin varlığında, serum ve idrar serbest hafif zincirlerin (FLC) ölçümü, pre analitik, analitik ve post-analitik problemlerden etkilenen Bence-Jones proteinüri (BJP) testi kullanımını sınırlayıp sınırlamayacağını değerlendirdik. Mart-Mayıs 2016 tarihleri arasında, plazma hücre diskrazisi şüphesi ile BJP testi için laboratuarımıza gönderilen 89 hasta değerlendirildi. MG varlığında, serum immünfiksasyon elektroforezi ile tiplendirilmiştir. Ek olarak, sFLC, uFLC ve ilişkili FLC-κ/λ oranı ve ayrıca glomerüler filtrasyon hızı (eGFR) ölçüldü. MG-κ'lı hastalar: ROC eğrisi analizleri, sFLC-κ ile sFLC-κ/λ oranına karşın BJP-κ pozitiflik deteksiyonu arasında doğru bir karşılaştırma olduğunu gösterdi. Üriner veriler, UFLC-κ karşın BJP-κ pozitiflik algılanması arasında iyi bir korelasyon gösterirken uFLC-κ/λ oranına karşın BJP-κ pozitiflik algılanması arasındaki korelasyon tatmin edici sonuçlar vermedi. MG-λ'lı hastalar: sFLC-λ ve sFLC-κ/λ oranına karşın BJP-λ pozitiflik algılanması karşılaştırıldığında, ROC eğrisi analizleri iyi korelasyon gösterdi. UFLC-λ ile BJP-λ pozitiflik deteksiyonu ve uFC-κ/λ oranına karşın BJP-λ pozitiflik algılanmasının üriner verileri, sensitivite ve spesifisite açısından tatmin edici sonuçlar vermedi. "κ" ve "λ" FLC'ye karşın BJP pozitiflik algılanması mutlak değerleri, istatistiksel olarak FLC-κ/λ oranından daha önemlidir. uFLC, [uFLC-Σ(κ+λ)] toplam verisi, BJP pozitifliği saptanması/algılanmasında istatistiksel olarak anlamlı bir medyan/ortalama değer tespit etti. Çalışma, aynı vakalarda BJP testi kullanımını optimize etme ihtimalini istatistiksel olarak göstermiş olmasına rağmen, tanımladığımız hedefleri doğrulamak için daha fazla geçerliliğe ihtiyaç vardır.

Anahtar Kelimeler: Plazma hücre diskrazisi, Myoklonal gamopati, Serum serbest hafif zinciri, İdrar serbest hafif zinciri, Bence-Jones proteinürisi

INTRODUCTION

In order to assemble the whole immunoglobulin, heavy chains (HCs) and light chains (LCs) synthesis takes place in an orderly manner, as well as their intracellular assembly.[1] As part of this process, low concentrations of free LCs (FLCs) are normally present in the serum of healthy subjects, but, when the LCs are compared to HCs production, physiologically they are produced in surplus.

The monoclonal sFLCs may be increased not only in plasma cell dyscrasias (PCDs)[2], but also in the B-cell lymphomas.[3,4]

In PCD not only is the kidney function often affected, but it is also imparied due to FLC catabolism that takes place exclusively in the kidney.[5] Kidney tubular abnormality is present in more than 98% of patients with PCD in whom Bence-Jones proteinuria (BJP) is > 1 g/24h; therefore, the tubular lesion associated with LCs overexcretion is always present.[6]

The peculiar metabolism of FLCs makes often the Bence-Jones proteinuria (BJP) detection complicated, because the same FLCs are part of the BJP. Moreover, the BJP detection is "sample-dependent".

The study was aimed to assess whether, in presence of monoclonal gammopathy (MG), regardless of plasma cell dyscrasia, the free light chains (FLCs) measurement, as well as FLC-κ/λ ratio, in both serum (sFLC) and urine (uFLC), may limit the BJP test employment.

MATERIAL AND METHODS

From March to May 2016, 122 patients who attended our laboratory for a suspected PCD were evaluated. For each patient, BJP and serum proteins electrophoresis (SPE) assays were performed. In case of monoclonal gammopathy (MG), in addition to SPE, serum immunofixation electrophoresis (IFE), serum and urine FLCs, as well as FLC-κ/λ ratio and alleged glomerular filtration rate (eGFR), by CK-EPI formula[7], were also measured. Serum and urine samples were stored at -80^{0}C and, for each sample, sFLC and uFLC in a single run were measured. Out of 122 patients recruited, 33 with eGFR < 60 mL/min were ruled out. Of the remaning 89 patients enrolled, 51 were males, aged between 33 and 83 years, median 65, and 38 were females, aged between 39 and 94 years, median 68.5.

The SPE were assessed utilizing Capillarys Protein(E) 6 instrument (Sebia®, Florence, Italy), whereas the IFE was performed on Hydragel 4 IF instrument (Sebia®, Florence, Italy). The BJP was detected by urine immunofixation electrophoresis (uIFEP) using a semi-automated agarose electrophoresis system (Hydragel 4 BJ and Hydrasys; Sebia®, Florence, Italy). For BJP detection, using Hydrasys kit, the sensitivity limit stated by the manufacturer is 50 mg/L. In order to improve the sensitivity, the samples may be concentrated 10 or 25 times, according to the proteinuria value.

In serum and urine, "κ" and "λ" FLCs were measured by means of Binding Site® kit (The Binding Site® Group Ltd., Birmingham, UK), (sFLC-k reference values (r.v.) 95th percentile= 3.3-19.4 mg/L; sFLC-λ r.v. 95th percentile= 5.7-26.3 mg/L; sFLC-k/λ ratio r.v.= 0.26-1.65, whereas uFLC-k r.v. 95th percentile= 0.39-15.1mg/L; uFLC-λ r.v. 95th percentile= 0.81-10.1 mg/L; uFLC-k/λ ratio r.v.= 0.461-4.00). After method implementation, the FLCs dosage was performed on an automatic analyzer[8], Olympus Au480 (Beckman Coulter®, Inc. CA, USA).

In Table 1 serum and urinary immunological features of 89 patients enrolled are summarized.

Statistical Analysis

Continuous data, as mean and standard deviation, or median and range, was reported. Binary data, as frequency and percentage values, ware also reported. Performance characteristics (sensitivity, specificity, Areas Under the Curves (AUC) and optimal cut-off for continuous variables) were evaluated by computing Receiver Operating Characteristic (ROC) curves.

Quantitative variables were compared using the non-parametric Mann-Whitney test or Student's t-test. A p-value ≤ 0.05 was considered statistically significant. The SPSS® (21.0) statistical program was used for all the analyses.

Table 1. Number of patients enrolled, number of patients with monoclonal gammopathy and BJP both detected and typified.

Number patients	MG	BJP-k Present	BJP-λ Present	BJP Absent
35	IgG-k	16	0	19
19	IgG-λ	0	4	15
3	IgM-k	1	0	2
2	IgM- λ	0	0	2
6	IgA-k	1	0	5
1	IgA- λ	0	0	1
9	Mixed (*)	1	1	7
14	Absent MG	0	0	14
Tot. 89		19	5	65

MG= Monoclonal Gammopathy; BJP= Bence-Jones proteinuria; (*) MG mixed = Simultaneous presence of 2 or 3 MG with same and/or different isotypes and light chains

The study, performed in accordance with the Declaration of Helsinki and complying with local laws, did not require informed consent or Ethics Committee approval because it was carried out employing samples obtained after routine analysis.

RESULTS

The measures of sFLC and uFLC and their correlation with BJP detection, whether it was present or absent, are summarized in Table 2.

Serum Data Analysis

sFLC-κ vs BJP-κ positivity detection in patients with monoclonal gammopathy-k

Taking into account the dosage of sFLC-κ vs BJP-k in 67 patients, including 44 patients with MG-k, 9 patients with mixed MG and 14 patients without monoclonal gammopathy, the areas under ROC curves (AUC) were 0.91 (95% I.C. : 0.82 - 0.99) (Figure 1). The sensitivity and specificity, with an optimal cut-off for sFLC-k dosage ≥ 45.7 mg/L, were 78.9% and 89.1%, respectively; the positive likelihood ratio (+LR) was 7.26.

Table 2. Summary of the serum and urine free light chain data vs BJP detection

FLC (mg/L)	BJP-k (n= 67)		BJP-λ (n= 45)	
	Absent (n= 48)	Present (n= 19)	Absent (n= 40)	Present (n= 5)
sFLC-k median (median range) (r.v. = 3.3 - 19.4 mg/L)	20.2 (3.2-90.5)	101.9 (14.2 - 2335)	15.9 (6.5 - 135.9)	6.1 (3.2 - 16.5)
sFLC-λ median (median range) (r.v. = 5.7 - 26.3 mg/L)	6.4 (3.0 - 23.6)	7.3 (2.7 - 180.8)	9.8 (3.0 - 104.4)	116.0 (77.3 - 7807)
sFLC-k/λ ratio median (median range) (r.v. = 0.26 - 1.65)	2.49 (1.07 - 30.2)	13.4 (2.12 - 778)	1.76 (0.3 - 10.47)	0.04 (0.0008 - 0.14)
uFLC-k median (median range) (r.v. = 0.39 - 15.1 mg/L)	14.4 (1.0 - 630.2)	175.3 (23.0 - 605.7)	10.7 (1.0 - 630.2)	10.2 (3.2 - 66.2)
uFLC-λ median (median range) (r.v. = 0.81 - 10.1 mg/L)	1.3 (0.3 - 77.8)	3.0 (1.1 - 37.3)	1.8 (0.6 - 7.80)	6.9 (2.3 - 32.1)
uFLC-k/λ ratio median (median range) (r.v. = 0.461 - 4.00)	8.46 (1.67 - 42.4)	50.1 (3.0 - 403.8)	7.1 (1.3 - 22.3)	1.4 (0.3 - 9.6)

FLC = Free Light Chain; sFLC = serum Free Light Chain; uFLC = urine Free Light Chain; BJP = Bence-Jones proteinuria)

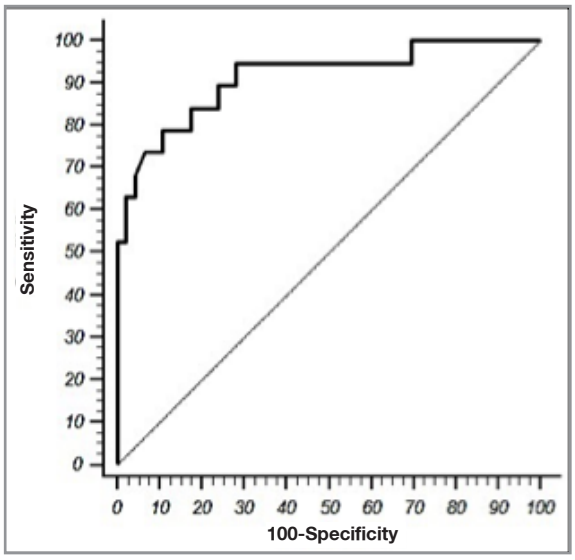

Figure 1. ROC curve analysis for sFLC-k dosages vs BJP-k detection. (Number of patients 67, considering patients with MG-k, patients with mixed MG and patients with MG absent. (MG = Monoclonal Gammopathy).

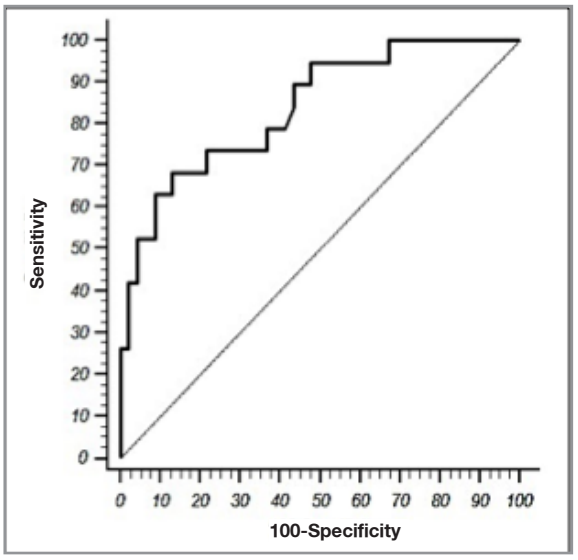

Figure 2. ROC curve analysis for sFLC-k/λ ratio vs BJP-k detection. (Number of patients 67, considering patients with MG-k, patients with mixed MG and patients with MG absent. (MG = Monoclonal Gammopathy).

sFLC-κ/λ ratio vs BJP-κ positivity detection in patients with monoclonal gammopathy-k

Always in 67 patients referred above, taking into consideration sFLC-κ/λ ratio vs BJP-κ positivity detection, the ROC curves showed an AUC = 0.84 (95% I.C. : 0.74 - 0.95) (Figure 2). The sensitivity and specificity, with an optimal cut-off for sFLC-κ/λ ratio ≥ 6.3, were 68% and 87%, respectively; +LR = 5.2.

sFLC-λ vs BJP-λ positivity detection in patients with monoclonal gammopathy-λ

Taking into consideration sFLC-λ vs BJP-λ positivity detection in 45 patients, including 22 patients with MG-λ, 9 patients with mixed MG and 14 patients without MG, the ROC curves showed an AUC = 0.99 (95% I.C. : 0.96-1.00) (Figure 3). The sensitivity and specificity, with an optimal cut-off for sFLC-λ dosage ≥ 39.7, were 100% and 97.1%, respectively; +LR = 35.0.

sFLC-κ/λ ratio vs BJP-λ positivity detection in patients with monoclonal gammopathy-λ

Always in 45 patients referred above, taking into consideration sFLC-κ/λ ratio vs BJP-λ positivity detection, the ROC curves showed an AUC = 1.0 (95% I.C. : 0.95 - 1.00) (Figure 4). The sensitivity and specificity, with an optimal cut-off for sFLC-κ/λ ratio ≤ 0.31, were 100% and 97.3%, respectively; +LR = 37.0.

Urine Data Analysis

uFLC-κ vs BJP-κ positivity detection in patients with monoclonal gammopathy-k

Comparing uFLC-κ vs BJP-κ positivity detection, always in 67 patients including patients with MG-k, mixed monoclonal gammopathy and patients without monoclonal gammopathy, as above reported, the ROC curves showed an AUC = 0.91 (95% I.C. : 0.82 - 1.00). The sensitivity and specificity, with an optimal cut-off for uFLC-κ dosage ≥ 52.6, were 88.9% and 85.7%, respectively; +LR = 6.2.

uFLC-κ/λ ratio vs BJP-κ positivity detection in patients with monoclonal gammopathy-k

In a comparative analysis between uFLC-κ/λ ratio vs BJP-κ positivity detection in 67 patients referred above, the ROC curves showed an AUC = 0.83 (95% I.C. : 0.64 - 1.00). The sensitivity and specificity, with an optimal cut-off for uFLC-κ/λ ratio ≥ 11.3, were 88.9% and 74.3%, respectively; +LR = 3.5.

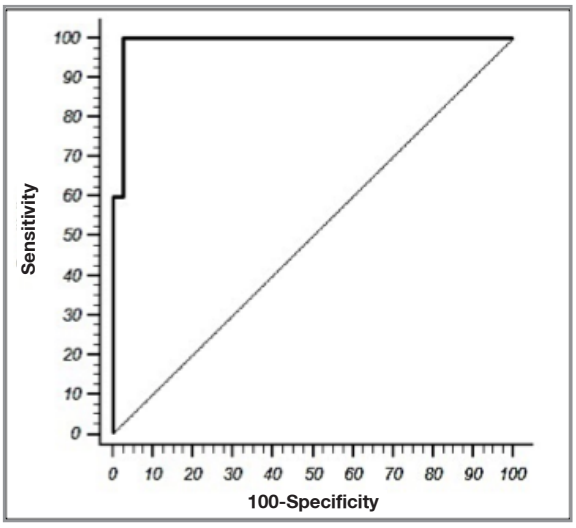

Figure 3. ROC curve analysis for sFLC-λ vs BJP-λ detection. (Number of patients 45, considering patients with MG-λ, patients with mixed MG and patients with MG absent. (MG = Monoclonal Gammopathy).

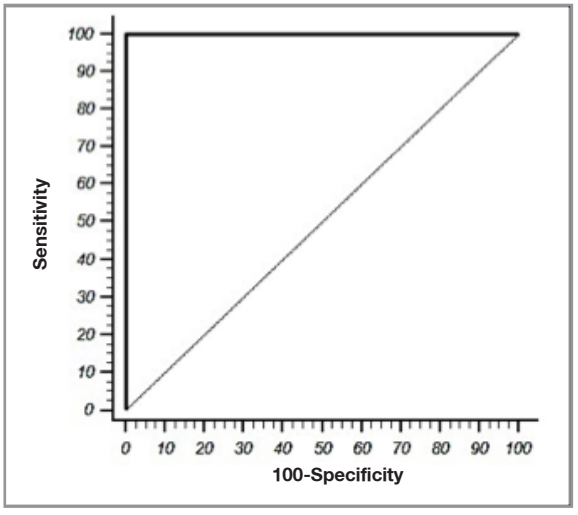

Figure 4. ROC curve analysis for sFLC-k/λ ratio vs BJP-λ detection. (Number of patients 45, considering patients with MG-λ, patients with mixed MG and patients with MG absent. (MG = Monoclonal Gammopathy).

uFLC-λ vs BJP-λ positivity detection in patients with monoclonal gammopathy-λ

When uFLC-λ data was compared with BJP-λ positivity detection in 45 patients, including patients with MG-λ, patients with mixed MG and patients without monoclonal gammopathy, the ROC curves showed an AUC= 0.82 (95% I.C. : 0.64 - 1.00). The sensitivity and specificity, with an optimal cut-off for uFLC-λ dosage ≥ 5.2, were 66.7% and 80.8%, respectively; +LR = 3.5.

uFLC-κ/λ ratio vs BJP-λ positivity detection in patients with monoclonal gammopathy-λ

Comparing uFLC-κ/λ ratio vs BJP-λ positivity detection, always in 45 patients referred above, the ROC curve showed an AUC= 0.73 (95% I.C.: 0.32 - 1.00). The sensitivity and specificity, with an optimal cut-off for uFLC-κ/λ ratio ≤ 1.5, were 66.7% and 96.2%, respectively; +LR= 17.6.

In Table 3 the summary of the statistical analysis is reported.

uFLC aggregated data, [uFLC-Σ (κ+λ)], vs BJP positivity detection.

As part of uFLCs analysis, the aggregated data, [uFLC-Σ (κ+λ)], vs BJP positivity detection was assessed. In presence of BJP detection, the median value of [uFLC-Σ (κ+λ)] was about 10 times higher compared to the median value found in patients in whom BJP was not detected, (p≤ 0.0001) (Table 4).

DISCUSSION

The FLC assay is a landmark test included in national and international guidelines[9,10] for diagnosis, prognosis and monitoring in the PCDs[11,12,13], as well as BJP test.

It's well known that the BJP test is affected by significant pre-analytical, analytical and post-analytical problems[14,15,16], therefore, its limited and rational employment in same cases could avoid the aforementioned problems.

Since FLCs catabolism takes place exclusively in the kidney, for this reason we believed that to recruit patients with normal eGFR was appropriate.[17,18]

The ROC curves analysis showed that comparing sFLC-κ and sFLC-κ/λ ratio vs BJP-κ positivity detection, the comparison was accurate (AUC= 0.91 and 0.84, respectively), with a +LR moderately useful (+LR= 7.26 and 5.25, respectively).

Table 3. Summary of statistical analysis between serum and urine FLC vs BJP detection

Monoclonal Gammopathy	sFLC and uFLC vs BJP detection	Cut-off (mg/L)	AUC	Sensitivity	Specificity	95% I.C.	+LR
MG-k	sFLC-k vs BJP-k						
	(n. patients 67)*	≥ 45.7	0.91	78.9	89.1	0.82 - 099	7.26
	sFLC-κ/λ ratio vs BJP-k						
	(n. patients 67)	≥ 6.3	0.84	68	87	0.74 - 0.95	5.2
	uFLC-k vs BJP-k						
	(n. patients 67)	≥ 52.6	0.91	88.9	85.7	0.82 - 1.00	6.2
	uFLC-κ/λ ratio vs BJP-k						
	(n. patients 67)	≥ 11.3	0.83	88.9	74.3	0.64 - 1.00	3.5
MG- λ	sFLC- λ vs BJP-λ						
	(n. patients 45)**	≥ 39.7	0.99	100	97.1	0.96 - 1.00	35.0
	sFLC- κ/λ ratio vs BJP-λ						
	(n. patients 45)	≤ 0.31	1.00	100	97.3	0.95 - 1.00	37.0
	uFLC- λ vs BJP-λ						
	(n. patients 45)	≥ 5.2	0.82	66.7	80.8	0.64 - 1.00	3.5
	uFLC- κ/λ ratio vs BJP-λ						
	(n. patients 45)	≤ 1.5	0.73	66.7	96.2	0.32 - 1.00	17.6

MG= Monoclonal Gammopathy; FLC= Free Light Chain; BJP= Bence-Jones proteinuria; AUC= areas under ROC curves; +LR= positive likelihood ratio. (*)Total number of patients with MG-k, mixed MG and MG absent. (**) Total number of patients with MG-λ, mixed MG and MG absent

With regards to the comparison between sFLC-λ and sFLC-κ/λ ratio vs BJP-λ positivity detection, the ROC curves analysis showed an AUC= 0.99 and 1.0, respectively.

Even the ROC curves analysis of urinary data showed a good correlation between uFLC-κ vs BJP-κ positivity detection (AUC= 0.91; +LR = 6.2), whereas the correlation between uFLC-κ/λ ratio vs BJP-κ positivity detection did not show satisfactory results (AUC= 0.81; +LR= 3.1).

Also urine analysis of uFLC-λ vs BJP-λ positivity detection and uFC-κ/λ ratio vs always BJP-λ positivity detection did not show satisfactory results for sensitivity and specificity (AUC= 0.82 and 0.73, respectively).

Concerning the samples with MG-λ and BJP-λ positivity, they were not representative; furthermore, this is supported epidemiologically by the detection of fewer patients with MG-λ compared with MG-κ patients.

Table 4. Comparison between aggregated data of uFLCs, [uFLC-Σ (κ+λ)], vs BJP detection

	BJP Absent (patients n. 65)	BJP Present (patients n. 24)	
uFLC-Σ (κ+λ) median (mg/L)	14.15	118.05	p≤ 0.0001

uFLCs = urine Free Light Chains; BJP = Bence-Jones proteinuria

The analysis of aggregate data of uFLC, [uFLC-Σ(κ+λ)], could be an interesting diagnostic feature in our point of view. It showed that the probability to detect BJP was statistically significant in patients with a median value 8 times higher than the value found in patients in whom BJP was not detected.

Our data still showed that the absolute values of "κ" and "λ" FLC, in both serum and urine, compared to the BJP positivity detection, statistically are much more significant than FLC-κ/λ ratio.

We still emphasize that the purpose of our study, according to the methodological setting, was principally to assess an optimal cut-off for sFLC and uFLC able to limit the BJP test employment in some cases in which monoclonal gammopathy is found. Although we identified statistically significant cut-offs able to optimize the detection of BJP positivity, further validations to confirm the objectives defined by our research are needed.

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