Original Article

Preliminary evaluation of concordance between Bence Jones protein and urine free light chain (FLC)

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Background: This preliminary study aimed to evaluate whether simultaneous measurement of urine-free light chain (FLC) with automated assay and Bence Jones protein (BJP) detection by urine-immunofixation (IFE) can provide additional clinical information in the management of patients with suspected monoclonal gammopathies (MG).

Methods: This retrospective study included 39 patients undergoing routine clinical analysis for urine FLC and BJP and other analytes. Their median age was 74.5 years [95% confidence interval (CI) for the median 70.92–81, range, 40–92 years], and 76.9% (30/39) of them were male. FLC measurements were performed with the N-Latex FLC method with the Atellica NEPH630 nephelometer (©Siemens, Marburg, Germany). The IFE for BJP detection was assessed using the Hydrasis instrument (Sebia, Evry Cedex, France).

Results: The presence of BJP was detected in nine urine specimens, seven samples with an FLC κ, and two with an FLC λ. The urine κ/λ ratio measured with the automated nephelometric assay was abnormal in ten urine samples. Results showed a good inter-rater agreement between BJP and urine κ/λ ratio results, with a weighted kappa (κ) coefficient of 0.65. Seven out of nine samples testing positive for the presence of BJP (77.8%) showed an altered κ/λ ratio, while two BJP-positive samples (22.2%) showed a normal κ/λ ratio (one sample with a FLC κ and one with FLC λ). Of the 10 samples with altered κ/λ ratio, only seven (70%) had a positive BJP.

Conclusions: These preliminary results suggest that, in patients with a known or suspected MG, the performance of both tests simultaneously does not provide additional information to those obtained by one test only.

Keywords: Monoclonal components (MC); immunofixation (IFE); free light chain (FLC); Bence Jones protein (BJP); monoclonal gammopathies (MG)

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Introduction

Identification and measurement of monoclonal components (MC) are fundamental parts of the diagnosis, management, and follow-up of monoclonal gammopathies (MG) and have been traditionally performed by electrophoresis and immunofixation (IFE) (1). Electrophoresis and IFE can be performed with serum and urine (2).

Analytical assays for free light chain (FLC) κ and λ became available several decades ago. The FLC measurement, at least in serum samples, represents an additional tool for the

assessment of patients with MG (3,4).

An abnormal κ/λ ratio, i.e., outside the reference interval, is proposed as a marker of clonality (1). It is also suggested that the automated measurement of serum FLC could replace Bence Jones protein (BJP) detection by IFE in urine specimens. However, in clinical conditions with minimal MC, the urine-IFI for BJP detection must be performed to reach the maximal diagnostic sensitivity (5).

Therefore, the aim of this preliminary study was to evaluate whether the simultaneous measurement of urine FLC with automated assay and BJP detection by urine-IFE can provide additional clinical information in the management of patients with suspected MG. Moreover, the possibility of replacing the search for BJP in IFE with a fully automated method, such as the nephelometric measurement of urine FLC, was investigated. We present this article in accordance with the STROBE reporting checklist (available at https://jlpm.amegroups.org/article/view/10.21037/jlpm-23-51/rc).

Methods

This retrospective preliminary study enrolled 39 consecutive patients undergoing routine clinical analysis for urine FLC and BJP and other analytes performed by the Clinical Biochemical Laboratory at the Hospital of Bolzano, Italy, during an 11-month period (from January 2022 to November 2022). The study group included 30 males and 9 females. The median age was 74.5 years [95% confidence interval (CI) for the median 70.92–81, range, 40–92 years] FLC measurements on urine spot samples were performed with the nephelometer Atellica® NEPH630 with the Latex-enhanced immunonephelometry N-Latex FLC method (Siemens, Marburg, Germany).

The IFE for BJP detection in urine non-concentrated spot specimens was performed with the instrument Hydrasis2 Scan® (Sebia, Evry Cedex, France), a compact multi-parameter system for agarose electrophoresis gels and IFE. All tests were performed following the manufacturer's instructions.

The FLC and urine κ/λ ratio concentration was considered abnormal when results were outside the reference interval provided by the manufacturers (urine κ/λ ratio 1.4–6.2). Unlike other providers, there were no different ranges based on kidney function.

This investigation was based on pre-existing data extracted from the laboratory information system in a fully anonymized form, so informed consent was unnecessary.

Qualitative concordance of the results obtained with FLC assays and urine-IFE for the detection of BJP was explored with the weighted kappa (κ) coefficient, where complete agreement was defined as κ coefficient =1.00, high agreement as 0.81≤ κ coefficient <1 and a good agreement when 0.61≤ κ coefficient <0.81. Concordance with diagnoses was analyzed when the results obtained with the two methods were not concordant, e.g., the presence of BJP and normal FLC measurement or *vice versa*. In our laboratory, serum electrophoresis was performed using Capillarys 2 Flex-Piercing® (Sebia, Lisses, France) and the serum IFI with Hydrasis2 Scan®. The kidney function in our laboratory was evaluated with the estimated-Glomerular Filtration Rate (GFR) using the Chronic Kidney Disease Epidemiology Collaboration (eGFR CKD-EPI) equation, based on the value of serum creatinine. This was obtained using a colorimetric kinetic test based on the Jaffè reaction (Roche cobas®, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis was performed with MedCalc17.4.4© statistical software (MedCalc Software, Ostend, Belgium). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Formal approval of the protocol by the local Ethics Committee was considered unnecessary because the study did not interfere with the usual clinical routine, as the assessment of FLC status was part of the routine examinations prescribed to patients after the acute event, and the data were handed anonymously. The data obtained from our study did not

Highlight box

Key findings

- The measurement of urine free light chain (FLC) does not bring additional advantages for the diagnosis of monoclonal gammopathies (MG) compared to serum and urine electrophoresis and immunofixation (IFE).

What is known and what is new?

- At the present, the measurement of urine FLC with automated assays is not included in the MG diagnostic and prognostic criteria.
- Report here about what does this manuscript adds.
- Our results, even if only preliminary, suggest that performing these two tests simultaneously not provide additional useful information in patients with a known or suspected MG.

What is the implication, and what should change now?

- The possibility to replace the search for Bence Jones protein in IFE by a fully automated method, such as the nephelometric measurement of urine FLC, need to be further investigated.

Table 1 Concordance between BJP and urine κ/λ ratio

Urine κ/λ ratio	BJP		
	Absence	Presence	Total
Normal	27	2	29 (74.4)
Abnormal	3	7	10 (25.6)
Total	30 (76.9)	9 (23.1)	

Data are presented as number or n (%). Weighted kappa 0.652; standard error 23.25; 95% CI: −1 to 1. BJP, Bence Jones protein; CI, confidence interval.

Table 2 Descriptive value of the measured urine FLC

Variables	Lowest value	Highest value	Median (95 % CI)	IQR
FLC κ mg/dL	4.6	736	49 (41.07 to 84.23)	61.97
FLC λ mg/dL	1.8	6310	22.5 (16.12 to 33.099)	26.75

FLC, free light chain; CI, confidence interval; IQR, interquartile range.

invalidate the patient's clinic. Given the retrospective data analysis nature, informed consent was not required.

Results

The presence of BJP was detected in nine urine specimens, seven samples with an FLC κ, and two with an FLC λ. The urine κ/λ ratio measured with the automated nephelometric assay was abnormal in 10 urine samples.

Inter-rater agreement between BJP and urine κ/λ ratio results showed a weighted kappa (κ) coefficient of 0.65 which represents a good agreement (see *Table 1*).

Seven samples, positive for the presence of BJP, showed an altered κ/λ ratio, while two BJP-positive samples showed a normal κ/λ ratio (one sample with a FLC κ and one with FLC λ). Of the ten samples with altered κ/λ ratio, only seven have a positive BJP.

The Shapiro-Wilk test for normal distribution rejected normality for the value measured for urine FLC κ and FLC λ ($P<0.0001$ for both). These values were reported as median and interquartile range (IQR). For FLC k the median was 49 mg/dL (95% CI: 41.07 to 84.23) and IQR 61.97 mg/dL. For FLC λ, the median was 22.5 mg/dL (95% CI: 16.12 to 33.099) and IQR 26.75 mg/dL (*Table 2*).

Patient number six (man, 52 years old) shows an abnormal κ/λ ratio (1.29) but absence of BJP. The measured FLC κ and λ were 83.8 and 64.5 mg/dL respectively (*Table 3*). We analyzed other laboratory data available for this subject. The serum electrophoresis and IFE did not reveal the presence of MC but a pattern of polyclonal hypergammaglobulinemia [1.97 g/dL, normal range (NR): 0.8–1.40 g/dL] was evident at electrophoresis and a decreased value of albumin (1.98 g/dL, NR: 3.8–4.75 g/dL) and beta-1 fraction (0.19 g/dL, NR: 0.4–0.7 g/dL) were detected. This patient showed a marked decrease in kidney function with an eGFR CKD-EPI of 8 mL/min 71.73 m^2. In this case, the abnormal κ/λ ratio did not appear to be secondary to an MG. Patient number 14 presented an abnormal urine κ/λ ratio (1.1) without a detected BJP. The FLC kappa is 89 and the FLC λ 80.4 mg/L. The eGFR CKD-EPI is 19 mL/min 71.73 m^2 (*Table 3*). The serum electrophoresis was the presence of a weak MC (0.03 g/dL) and at IFE a CM immunoglobulin (Ig)G kappa was detected. In this case, the abnormal urine κ/λ ratio is secondary to an MG. However, the measurement of urine FLC was irrelevant because the CM was already identified by electrophoresis, IFE, and serum FLC κ/λ ratio. Patient 32 showed a normal serum electrophoresis, serum IFE did not reveal the presence of MC, and absence of BJP but an abnormal serum κ/λ of 0.29 was observed (reference interval provided by the manufacturers for serum κ/λ ratio: 0.31–1.56). The eGFR CK EPI is 84 mL/min 71.73 m^2. In this case, the abnormal urine κ/λ ratio did not appear to be secondary to an MG, and a final diagnosis of an isolated inflammatory lesion of the right cerebellar peduncle of a post-infectious nature was also reported. Patient 30 shows a BJP κ and the presence of an MC at the serum electrophoresis (0.56 g/dL and an MC IgM λ at the serum IFE). In this case, the urine κ/λ ratio showed normal despite the presence of an MG (*Table 3*).

Discussion

At present, the measurement of urine FLC with automated assays is not included in the MG diagnostic and prognostic criteria (3). The possibility to measure FLC either in serum and urine with automated assays has provided additional tools in the management of MG. The detection of BJP by IFE requires trained operators and is a time-consuming method. Fully automated assays represent an intriguing alternative for clinical laboratory specialists, but the real utility of the new assay in the management of different diseases needs to be demonstrated by clinical studies.

Recent studies have reported on the need for the serum FLC measure in the MG diagnosis-showing that κ/λ ratio may be abnormal in some patients without MG. This is

Table 3 Patients with both positive results at the BJP detection and abnormal urine κ/λ ratio (7 patients), negative BJP and abnormal urine κ/λ ratio (3 patients) and positive BJP detection and normal urine κ/λ ratio (1 patient)

Patient No.	BJP	Urine κ/λ ratio	Urine FLC κ	Urine FLC λ	Gender and age, years
Patient 6	Neg	1.29	83.8	64.5	Male, 52
Patient 14	Neg	1.1	89	80.4	Female, 80
Patient 32	Neg	0.82	45.1	54.9	Male, 43
Patient 30	Pos λ	4.24	170	40.3	Male, 67
Patient 3	Pos λ	0	49	6,310	Female, 71
Patient 9	Pos κ	12.68	255	20.1	Female, 82
Patient 16	Pos κ	1.18	47.4	40.1	Male, 61
Patient 18	Pos κ	6.81	36.8	5.4	Male, 81
Patient 19	Pos κ	79.13	736	9.3	Female, 92
Patient 25	Pos λ	0.41	440	1,070	Male, 77
Patient 38	Pos κ	10	225	22.5	Male, 82

Urine κ/λ ratio reference interval: 1.4–6.2. BJP, Bence Jones protein; FLC, free light chain; Neg, negative; Pos, positive.

certainly true for hyper gamma polyclonal globulinemia (6) as in patients 6 and 32 of our study. The behavior of urine FLCs in hypergammaglobulinemia and renal failure cases is not fully understood. Xu *et al.* recently reported that urine FLC showed an increase that is proportional to the decrease of renal function by the N-Latex FLC method, but with a weak correlation and a relatively stable urine κ/λ ratio (within the reference interval provided by the manufacturers) while the κ/λ ratio obtained with another method was strongly affected by renal failure (7). However, a high false negative rate for serum κ/λ ratio in patients with GM has been reported (8). Although limitations on the use of FLC in the diagnosis of GM were reported, the measurement of serum FLC is recommended by guidelines (3). In contrast, the measurement of urinary FLC is not recommended by current guidelines, which instead recommend the searching of BJP research with urine-IFE.

The aim of this preliminary study was to evaluate whether concomitant measurement of urine-FLC and BJP could provide additional information in patients with or suspected MG. Our results, even if only preliminary, suggest that performing these two tests simultaneously does not provide additional useful information in patients with a known or suspected MG. These results cannot be generalized because there are different methods on the market for measuring FLC (9,10). Recently, some authors have stressed the need to verify the reference intervals for the serum κ/λ ratio, as recommended by the international guidelines (11-13). In our opinion, the reference ranges for urine κ/λ ratio provided by different manufacturers must also be applied. Another aspect to be considered is that in this study the results from spot urine samples were used and different results could be obtained using 24 hours of collected urine samples. The use of concentrated or non-concentrated urine samples for BJP research could also be an additional source of variability.

Conclusions

Our conclusions are in line with those reported by other authors, showing that the measurement of urine FLC does not bring additional advantages for the diagnosis of GM compared to serum and urine electrophoresis and IFE (14). The main limit of our preliminary study is the small number of the patients included and obviously, our conclusion must be validated by further studies.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://jlpm.amegroups.org/article/view/10.21037/jlpm-23-51/rc

Data Sharing Statement: Available at https://jlpm.amegroups.org/article/view/10.21037/jlpm-23-51/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jlpm.amegroups.org/article/view/10.21037/jlpm-23-51/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Formal approval of the protocol by the local Ethics Committee was considered unnecessary because the study did not interfere with the usual clinical routine, as the assessment of FLC status was part of the routine examinations prescribed to patients after the acute event, and the data were handed anonymously. The data obtained from our study did not invalidate the patient's clinic. Given the retrospective data analysis nature, informed consent was not required.

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