# The detection of Bence Jones protein in urine by the heat test helps in diagnosis of multiple myeloma?

*A detecção de proteína Bence Jones na urina pelo teste de calor auxilia no diagnóstico de mieloma múltiplo?*

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## ABSTRACT

**Introduction:** Multiple myeloma (MM) is a hematologic malignancy caused by the intense indiscriminate proliferation of plasma cells in the bone marrow. In view of clinical suspicion of MM, clinic laboratory tests and imaging tests should be used, among others. **Objectives:** Evaluate the laboratory test for protein detection heat method Bence Jones (BJ) used to complementary diagnosis pathology and to characterize the epidemiological profile of patients diagnosed with MM. **Material and methods:** A retrospective study was conducted from January 2010 to July 2015 of the patients treated at Hospital de Clínicas of Universidade Federal do Paraná (HC/UFPR) in Curitiba, Paraná, Brazil. **Results:** In the patients analyzed, the average age at diagnosis of MM was 65.6 years, with a minimum percentage of difference between genders [males 52.6% ($n = 10$) and females 47.4% ($n = 9$)], predominantly in the white race [84.2% ($n = 16$)]. Among the patients analyzed, 85.2% ($n = 104$) had negative BJ exam and 14.8 ($n = 18$), positive exam; 84.4% ($n = 103$) had no diagnosis of MM, and 15.6% ($n = 19$) were diagnosed with the disease. **Conclusion:** The evaluation results of BJ protein detection by the heat method showed sensitivity of 47.4%, specificity of 91.3%, with positive and negative predictive values of 50% and 90.4%, respectively.

**Key words:** Bence Jones protein; multiple myeloma; urine.

## INTRODUCTION

Multiple myeloma (MM) is a hematologic malignancy caused by the intense indiscriminate proliferation of plasma cells in the bone marrow leading to overburden and suppressing the production of other cells. As a consequence, immunoglobulins and their fragments accumulate in peripheral blood[1].

The excess of immunoglobulin light chains is filtered at the glomerulus and appears in the urine, being called monoclonal proteins of Bence Jones (BJ). These proteins are damaging and toxic to tubular cells and lead to renal tubular dysfunction, thus contributing to the development of chronic renal failure[2].

Although the presence of the protein in urine was firstly reported in 1847 by Dr. Henry Bence Jones, and the disease was completely described in 1850 by Dr. MacIntyre[3], so far the etiology of MM remains unknown. However, increased frequency was observed in individuals exposed to irradiation, wood, leather, and oil by-products, besides in farm laborers[4].

The physiopathological implications of MM in advanced stage are bone destruction, kidney failure, suppression of hematopoiesis and increased risk of infections[5].

Patients with MM can remain asymptomatic for a long period, what contributes to late diagnosis. Laboratory exams are of great relevance in these cases, because they provide the opportunity for early diagnosis and adequate treatment, improving patients' survival[6].

## OBJECTIVES

The present study proposes a retrospective analysis for the determination of sensitivity and specificity of the BJ technique

used as complementary diagnosis of MM, as well as the analysis of data such as gender, age, and race, in order to characterize the epidemiological profile of patients diagnosed with the disease.

## MATERIAL AND METHODS

### Case analysis

One hundred twenty-two patients were analyzed who underwent the exam of detection of BJ urinary protein from January 2010 to July 2015, at the laboratory of bacteriology and urinalysis of Hospital de Clínicas of Universidade Federal do Paraná (HC/UFPR). For the diagnosis of MM, the tests of bone marrow aspirate and/or biopsy were considered gold standard in the studied period. The project was evaluated and approved by the research ethics committee of the institution (CAAE: 50977315.4.0000.0096).

### Technique

Urine (10 ml) was added to a glass test tube to be analyzed. The sample was acidified to pH 5.0 with 3% sulfosalicylic acid. Then the tube was put in a boiling water bath for 5 minutes and the sample was filtered while still hot. In case turbidity or precipitation was visualized during cooling, the tube was put again in the boiling water bath. The research was considered positive if precipitation disappeared at 100ºC and reappeared with cooling.

### Statistical analysis

Results of quantitative variables were described as means, medians, minimum values, and maximum values. The qualitative variables were described as frequencies and percentages. In order to assess sensitivity and specificity of the BJ method, the diagnosis of MM was considered gold standard. Values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were estimated. Data were analyzed with the software IBM SPSS Statistics v.20.

## RESULTS

In the bone marrow examination (aspirate) and in the biopsy, the 19 patients presented results compatible with MM, with plasma cells greater than 10%. Among the 19 patients diagnosed with MM, there was a percentage in the male gender of 52.6% ($n = 10$) and in the female of 47.4% ($n = 9$), predominantly in the white race

[84.2% ($n = 16$)]. Patients' age ranged from 56 to 90 years, with mean and median of 66.9 and 65 years, respectively.

In the total of 122 analyzed patients, 85.2% ($n = 104$) presented negative BJ test (not detected) and 14.8% ($n = 18$), positive result (detected). Distribution of BJ results regarding the presence of MM is shown in the **Table**.

The analysis of statistical significance among the results of protein BJ in the detection of MM obtained sensitivity of 47.4%, specificity of 91.3%, PPV and NPV of 50% and 90.4%, respectively.

TABLE − Distribution of BJ investigation among patients regarding MM

| BJ | Diagnosis | |
|---|---|---|
| | Without MM | With MM |
| Negative ($n = 104$) | 94 (91.3%) | 10 (52.6%) |
| Positive ($n = 18$) | 9 (8.7%) | 9 (47.4%) |
| Total | 103 | 19 |

BJ: Bence Jones; MM: multiple myeloma.

## DISCUSSION

MM accounts for 1% of all neoplasms and 10% of hematological neoplasms, being more common in elderly patients[7]. In the current research, patients' age ranged from 56 to 90 years (mean of 66.9 years) and a percentage of minimal difference was noted between genders: it was more often in males (52.6%), predominating in the white race [84.2% ($n = 16$)]. Soleymanian et al. (2016)[8] evaluated 57 patients with MM and obtained an average age of patients from 35 to 80 years, with prevalence in the male gender (65%). However, Sandy Jr. et al. (2015)[9] highlighted the importance of not disregarding suggestive clinical findings, even in patients out of the age group affected by the disease, and they stressed the relevance of case reporting, since in the literature just 0.3% of the described cases occurred in younger patients. In the current study, the disease predominated in the white race. Studies conducted by Silva et al. (2009)[5] confirmed the obtained results that demonstrated higher prevalence in this race.

Although the BJ protein detection is a simple test to be carried out in clinical laboratories, it presents limitations[2, 10]. Concerning the diagnosis of MM, the method used in this research resulted in high specificity (91.3%), but low sensitivity (47.4%). Some researchers revealed that the heat detection method can present false positive results when there is an excess of polyclonal proteins in the sample, with low specificity, sensitivity and reproducibility. In these cases, researchers recommend the conduction of immunofixation electrophoresis of proteins in the urine[11, 12].

In the present study, nine (8.7%) patients with positive BJ did not have MM and presented different diagnoses, such as non-Hodgkin lymphoma, lupus, pneumonia, and bladder cancer.

The presence of BJ protein is associated mainly to MM, but other diseases can present positive results, such as Waldenstrom macroglobulinemia, amyloidosis, and light-chain deposition disease. Patients with lymphoma, chronic lymphocytic leukemia, and monoclonal gammopathy of undetermined significance are also reported[13]. BJ protein can also be present in lymphoid tumors, non-lymphoid cancer, and non-neoplastic conditions, such as cirrhosis, sarcoidosis, parasitic diseases and autoimmune disorders[14].

Among 10 patients (52.6%) that presented MM, BJ investigation was negative. Strasinger and Di Lorenzo[10] reported that not all individuals with MM excrete detectable levels of BJ protein in the urine, what produces false negative results.

Jenner[15], in 2004, performed a study comparing the measurement of serum free chains between electrophoresis of serum and urinary proteins, immunofixation and detection of BJ protein. In its explanation, the light chain concentrations are filtered by the kidneys and progressively increase in serum before appearing in the urine; in cases of low concentrations of proteins, there will be no adequate concentration for urinary detection, considering that the BJ test is not a direct reflex of production or detection of underlying monoclonal protein. The same applies to electrophoresis exam: detection of low-level proteins may not evidence the peak in the corresponding band; in contrast, samples with high concentration may give the false impression of monoclonality and heavy proteinuria, containing polyclonal chains and making interpretation difficult. Due to the stressed limitations, the international guidelines suggest that serum free chain test is replaced by urine and serum electrophoresis in the diagnosis of monoclonal gammopathies.

## CONCLUSION

MM progresses silently. Many times, when patients search for treatment, an advanced condition of severe bone lesions and/or kidney failure is installed. The BJ protein detection technique is a method easy to conduct and of low cost still used in clinical laboratories. However, data from the research demonstrated that it does not contribute effectively to the diagnosis of MM, and it must be replaced by more sensitive and specific techniques, such as urine and serum electrophoresis.

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## RESUMO

*Introdução: O mieloma múltiplo (MM) é uma neoplasia maligna hematológica causada pela intensa proliferação indiscriminada de plasmócitos na medula óssea. Diante da suspeita clínica de MM devem ser realizados exames laboratoriais e exames de imagem, entre outros. **Objetivos:** Avaliar o teste laboratorial de calor para detecção de proteína Bence Jones (BJ), utilizado como diagnóstico complementar da patologia, e caracterizar o perfil epidemiológico dos pacientes diagnosticados com MM. **Material e métodos:** Foi realizado um estudo retrospectivo de janeiro de 2010 a julho de 2015 dos pacientes atendidos no laboratório do Hospital de Clínicas da Universidade Federal do Paraná (UFPR), Curitiba, Paraná, Brasil. **Resultados:** Dos pacientes avaliados, a média de idade no momento do diagnóstico de MM foi de 65,6 anos, com um percentual de diferença mínima entre os gêneros masculino [52,6% (n = 10)] e feminino [47,4% (n = 9)] e predomínio na raça branca [84,2% (n = 16)]. Entre os pacientes analisados, 85,2% (n = 104) apresentaram exame de BJ negativo e 14,8 (n = 18), positivo; 84,4% (n = 103) não apresentaram diagnóstico de MM e 15,6% (n = 19) foram diagnosticados com a patologia. **Conclusão:** Os resultados da avaliação do método de calor de detecção de proteína BJ mostraram sensibilidade de 47,4% e especificidade de 91,3%, com valores preditivos positivo e negativo de 50% e 90,4%, respectivamente.*

*Unitermos: proteína de Bence Jones; mieloma múltiplo; urina.*

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