2012 © Curr. Issues Pharm. Med. Sci. Vol. 25, No. 4, Pages 430-433

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy

Blood serum free light chain concentration vs. immunofixation results in patients with monoclonal gammapathy

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ABSTRACT

Monoclonal gammapathies are a group of diseases characterized by proliferation of plasma clone cells that produce a certain type of homogenic immunoglobulin containing heavy chains of α, γ, μ, δ, ε class aside a type of light κ or λ chains. Standard methods applied to detect and identify the type of monoclonal protein in patients suspected of monoclonal gammapathy include serum electrophoresis and immunofixation. Quantitative analysis of free light chains (FLCs) by nephelometry has become more popular recently. The purpose of the study was to compare the results and assess the compatibility of free light chains measurements obtained by nephelometry and standard immunofixation. In addition to that, we tried to evaluate the usefulness of serum FLC analysis in the detection of monoclonal protein. The investigation was carried out in the group of 92 patients with suspected monoclonal gammapathy. In most cases concentrations of FLCs are increased depending on the type of light chain determined by immunofixation, however normal values of FLCs and normal kappa/lambda ratio do not exclude the presence of monoclonal protein. The analysis of FLC is most useful to identify patients suspected of light chain disease. Immunofixation is more sensitive method to detect gammapathies with complete monoclonal protein in comparison to quantitative FLC analysis.

Keywords: free light chains, monoclonal gammapathy, immunofixation

INTRODUCTION

Monoclonal gammapathies are a group of diseases characterized by proliferation of plasma clone cells that produce a certain type of homogenic immunoglobulin containing heavy chains of α, γ, μ, δ, ε class aside a type of light κ or λ chains. Immunoglobulins produced by plasmocyte clone are called monoclonal proteins, most frequently of IgG or IgA class. In light chain disease (LCD) monoclonal protein contains only one type light chain and in case of heavy chain disease (HCD) it contains heavy chains only [4,9,10].

Standard methods applied to detect and identify the type of monoclonal protein in patients suspected of monoclonal gammapathy include serum electrophoresis and immunofixation. Of those two immunofixation is more sensitive [2,19].

Quantitative analysis of free light chains (FLCs) by nephelometry has become more popular recently. The production of FLCs by plasmocytes and B lymphocytes, that also account for the production of a heavy chain type, is 40% higher than the quantity of heavy chains [18]. The analysis of FLC has become useful to monitor treatment of patients with plasma cell myeloma (myeloma multiplex), amyloidosis [13], LCD [8] and non-secretory myeloma [3]. Based on the scores of light chains it is possible to calculate kappa/lambda ratio useful to differentiate between monoclonal and polyclonal gammapathy [6]. Concomitant rise in kappa and lambda chain concentrations is observed in autoimmune diseases and also in renal failure in the course of which kappa/lambda ratio remains normal [2]. In case of monoclonal gammapathies kappa/lambda chain ratio is impaired as a result of overproduction of one chain type over the other [6,8]. Moreover, abnormal kappa/lambda ratio is an independent progression risk factor in monoclonal gammapathy of undetermineded significance (MGUS), so called "smoldering" plasma cell myeloma and solitary plasmocytoma [12,16].

The purpose of the study was to compare the results and assess the compatibility of free light chains measurements obtained by nephelometry and standard immunofixation. In addition to that, we tried to evaluate the useful-

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ness of serum FLC analysis in the detection of monoclonal protein.

MATERIAL AND METHODS

Examination group. The investigation was carried out in the group of 92 patients with suspected monoclonal gammapathy: 50 females (mean age 65yrs) and 42 males (mean age of 64yrs). In this group serum monoclonal proteins were detected by electrophoresis and immunofixation in 81 patients. The majority of them had myeloma type with complete monoclonal protein: IgG in 44 patients, IgA in 11 patients and IgM in 4 patients. In 18 patients blood serum monoclonal component was formed solely of light chains, in 4 patients immunofixation detected oligoclonal protein and in 11 patients no monoclonal proteins were found. The patients were treated in the Outpatient Clinic of Hematooncology and Bone Marrow Transplantation and The Clinic of Hematooncology, Autonomous Public University Hospital No.1(SPSK-1) in Lublin.

Methods. Blood serum samples were taken from the patients fasting from the cubital vein to the test tubes without anticoagulants. The test tubes were centrifuged for 10min at 2,000rpm. Thus obtained serum underwent testing by electrophoresis, immunofixation and free light chains of kappa and lambda type were determined. The testing was carried out in The Unit of Laboratory Diagnostics, SPSK-1 in Lublin.

Electrophoresis of blood serum proteins was performed by Hydragel Protein 15/30 kit (Horiba ABX Diagnostics) adjusted to automatic Hydrasys system. Electrophresis was done on agarose gels. The method allows for detecting five blood serum fractions. Separated fractions were stained with amido black and electrophoregrams were read by a scanner PHORESIS 4.1x software.

Immunofixation was done by Hydragel 4IF kit (Horiba ABX Diagnostics). The testing used antiserums against heavy chains (anti-IgG, anti-IgM, anti-IgA) and antiserums against light chains (anti-κ and anti-λ).

Free light chains were analyzed by FREELITE ™ kit (The Binding Site) by nephelometric technique using Siemens BN II. FREELITE ™ reagent kit is designed for quantitative determination of free light chains. The antibodies used are epitope-specific, accessible only in kappa and lambda free light chains, which guarantees detection of free light chains only. The reference values for the investigated immunoglobulin chains ranges are: FLC κ 3.3-19.4 mg/L; FLC λ 5.71-26.3 mg/L; FLC κ/λ 0.26-1.65.

Statistical analysis. The results were statistically analyzed by STATISTICA 10 (StatSoft). The nephelometric results for kappa and lambda scores that grouped beyond the reference range and immunofixation results within the reference range depending on kappa or lambda bands present/absent were presented as contingency tables obtained by Chi^2 test. The differences and correlations were considered statistically significant at $p<0.05$.

RESULTS

Table 1 presents serum free light chains of kappa type and kappa/lambda ratio depending on the type of monoclonal protein obtained by immunofixation.

Table 1. Blood serum FLC of kappa type in 42 patients with kappa band, free or bound with heavy chain obtained by immunofixation

IF (immunofixation)	n	Patients with abnormal kappa FLC n (%)	Patients with abnormal kappa/lambda n (%)
IgG κ	27	22 (81.25)	20 (74)
IgA κ	6	5 (83)	5 (83)
IgM κ	1	1(100)	1 (100)
κ FLC	8	8 (100)	8 (100)
Total	42	36 (85.7)	34 (80)

n – number of patients

In the group of 42 patients who had monoclonal component built of kappa FLC, free or bound with heavy chains determined by immunofixation 80% patients had abnormal kappa/lambda ratio. The biggest group included the patients with monoclonal protein of IgG kappa type, of whom 81.2% had improper kappa-FLC values and 74% had improper kappa/lambda ratio. All patients with free kappa band on immunofixation had increased blood serum concentrations of FLC and abnormal kappa/lambda ratio.

Table 2 presents blood serum FLC of lambda type and kappa/lambda ratio depending on the type of monoclonal protein obtained by immunofixation.

Table 2. Blood serum FLC of lambda type in 35 patients with lambda band, free or bound with heavy chain obtained by immunofixation

IF (immunofixation)	n	Patients with abnormal lambda FLC n (%)	Patients with abnormal Kappa/Lambda n (%)
IgG λ	17	8 (47)	9 (53)
IgA λ	5	3 (60)	4 (80)
IgM λ	3	2 (66)	0 (0)
λ FLC	10	9 (90)	9 (90)
Total	35	22 (62)	22 (62)

n – number of patients

In the group of 35 patients who had monoclonal protein built of lambda FLC, free or bound with heavy chains determined by immunofixation 62% had abnormal kappa/lambda ratio. Kappa/lambda ratio was abnormal in 53% patients with monoclonal IgG lambda protein present. However, in the group of patients with free lambda band detected, 90% patients had abnormal FLC of lambda type and significantly altered kappa/lambda ratio.

In addition to that we tried to assess the compatibility of FLC lambda and FLC kappa scores determined by nephelometry and by standard immunofixation used to identify monoclonal protein (tables 3 and 4).

Table 3. Correlation between FLCs of kappa type and their scores on immunofixation used to identify light chains of kappa type, free or bound with heavy chains

Band on kappa pathway (IF)	Kappa (mg/l) - beyond range	Kappa (mg/l) -within range	Total
Present (+)	36	6	42
%line	85.7	14.3	
Absent (-)	12	34	46
%line	26	74	
Total	48	40	88

p<0,001 Compatibility 79,5%, sensitivity 85.7%, specificity 74%

The results of kappa-FLC are 79,5% compatible however they have sensitivity and specificity of 85.7% and 74% respectively with reference to immunofixation which detected a band on kappa pathway (Table 3). In the group of 42 patients with kappa chain on immunofixation 6 patients had normal kappa FLC (3.3-19.4 mg/L) and kappa/lambda ratio (0.26-1.65).

Table 4. Correlation between FLCs of lambda type and their scores on immunofixation used to identify light chains of lambda type, free or bound with heavy chains

Band on lambda pathway (IF)	Lambda (mg/l) – beyond range	Lambda (mg/l) – within range	Total
Present (+)	26	9	35
%line	74.3	25.7	
Absent (-)	1	52	53
%line	1.8	98.2	
Total	27	61	88

p<0,001 Compatibility 88.6 %; sensitivity 74 %; specificity 98%.

The results of lambda-FLC are 88.6% compatible however they have sensitivity and specificity of 74% and 98% respectively with reference to immunofixation, which detected a band on lambda pathway (Table 4). In the group of 35 patients with lambda chain on immunofixation 9 patients had normal lambda FLC (5.71-26.3 mg/L) and kappa/lambda ratio (0.26-1.65).

DISCUSSION

The investigation demonstrated how new quantitative analysis scores of free light chains correlate with the results obtained by standard immunofixation used to detect monoclonal proteins, complete or formed of free light chains. We assessed possible usefulness of quantitative determinations of free light chains to detect monoclonal protein.

Jaskowski et al. [5] found that the concentrations of kappa free light chains were abnormal in 72.9% patients who had a band detected on kappa pathway by immunofixation. When lambda band was detected on immunofixation, the concentrations of lambda free light chains were beyond the range of reference in 91.4% patients.

Our results also confirmed increased FLC concentrations of either kappa or lambda chain on immunofixation. When kappa band was detected on immunofixation, the values were beyond the reference range in 85.7% patients. When lambda FLC was detected by immunofixation the concentrations of lambda FLC were increased in 62% patients.

The results suggest that excessive production of FLC was noted in the majority of patients in whom monoclonal protein was detected on immunofixation. In a small group of patients FLC concentrations were within normal range (14.3% and 25.7% respectively) despite the detection of kappa or lambda band on the pathway, which suggests that no overproduction of FLC occurred. Those results lowered the compatibility level between the results of quantitative analysis and immunofixation, which were 79,5% and 88.6% for kappa and lambda free light chains respectively. Our test compatibility results were lower than presented by Jaskowski et al. [5], which can be accounted for by a smaller group of examined patients.

Abraham et al. [1] found free lambda band and increased FLC concentrations on immunofixation in all patients who had LCD diagnosed. In 89% patients with LCD and kappa band confirmed on immunofixation, FLC concentrations were higher than the normal reference range. Our own results support that finding, too. Kraj et al.[11] obtained similar results. They confirmed very good sensitivity of serum FLC measurements in patients with LCD. All cases of LCD were diagnosed appropriately based on quantitative analysis of serum FLCs by nephelometry.

Another parameter compared with immunofixation results was kappa/lambda ratio, which seems to have bigger diagnostic value than FLC determined alone. Singhal et al. [17] found abnormal kappa/lambda values in 66% patients who tested positively on immunofixation. Our results confirm that finding, too. We found abnormal values of kappa/lambda ratio in 73% patients who had kappa or lambda band detected on immunofixation. Jaskowski et al. [5] found abnormal values of kappa/ lambda in 32% patients who tested positively on immunofixation. Many other authors reported on abnormal kappa/lambda ratio frequently noted in patients with suspected LCD [7,11,19,20].

Singhal et al. [17] also noted that normal kappa/lambda ratio may signal elimination of paraprotein despite positive immunofixation results; Mead et al. [14] confirmed that finding, too. Mösbauer et al. [15] found that the reduction of sFLCs preceded negative immunofixation by 128 days mean, and in patients with disease recurrence increased concentrations of sFLCs by 25% were noted 98 days prior to the detection of monoclonal protein on immunofixation. However chronically abnormal kappa/lambda ratio may precede complete monoclonal protein detected by immunofixation [17,18].

CONCLUSION

1. Immunofixation is more sensitive method to detect gammapathies with complete monoclonal protein in comparison to quantitative FLC analysis.

2. The analysis of FLC is most useful to identify patients suspected of light chain disease.
3. In most cases concentrations of FLCs are increased depending on the type of light chain determined by immunofixation, however normal values of FLCs do not exclude the presence of monoclonal protein.
4. Normal kappa/lambda ratio does not exclude plasma cell dyscrasia with monoclonal proteins produced in its course.

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