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Concentrations of free light chains determined by nephelometry and turbidimetry

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ABSTRACT

Determination of FLCs in the blood serum has been useful to diagnose patients with monoclonal gammapathies. Besides, the test has been widely used to assess response to treatment and detect progressive conditions. In the course of gammapathy monoclonal light chains constitute homogenic population of chains made of kappa and lambda light immunoglobulins produced by malignant plasmocytes and/or B lymphocytes. Quantitative analysis of FLC by nephelometry and turbidimetry uses specific antibodies which recognize antigenic determiners of light chains. The purpose of our investigation was to compare nephelometric and turbidimetric methods applied in FLC determinations. Comparative determinations were done in the group of 64 patients suspected of monoclonal gammapathy: 30 females (mean age 66yrs) and 34 males (mean age 64yrs). The analysis of results let conclude that nephelometric and turbidimetric techniques applied to determine kappa and lambda free light chains have comparable diagnostic value.

Keywords: free light chains, nephelometry, turbidimetry, monoclonal gammapathy

INTRODUCTION

Determination of blood serum free light chains (FLCs) was introduced into hematological practice in the USA in 2001 [2]. Kappa and lambda light chains are synthesized at defined rate and surplus over heavy chains by plasmocytes and B lymphocytes in the bone marrow and lymph nodes. Biological half life time of kappa and lambda FLCs is 2-4hrs and 3-6hrs respectively. Moreover, the synthesis of kappa monomer chains is two times bigger than dimmer lambda chains.

In healthy persons FLCs are filtered in the renal glomeruli and metabolized in the proximal tubules. Physiologically only small quantities of FLCs are excreted with urine (1-10mg/24hrs). Most probably they are secreted via mucus membrane of the last section of the nephrone and ureter [4,6,10,12].

Determination of FLCs in the blood serum has been useful to diagnose patients with monoclonal gammapathies. Besides, the test has been widely used to assess response to treatment and detect progressive conditions. In the course of gammapathy monoclonal light chains constitute homogenic population of chains made of kappa and lambda light immunoglobulins produced by malignant plasmocytes and/or B lymphocytes [2,13].

Quantitative analysis of FLC by nephelometry and turbidimetry uses specific antibodies which recognize antigenic determiners of light chains, which in complete immunoglobulin are covered by adjacent heavy chain. Latex balls coated with antibodies are used to enhance sensitivity of analysis. FLC analysis, which has become a common diagnostic practice recently, is most frequently done by nephelometric method [4,6,9].

There are only few reports in literature that compare various quantitative analyses of FLCs. The purpose of our investigation was to compare nephelometric and turbidimetric methods applied in FLC determinations.

MATERIAL AND METHOD

Comparative determinations were done in the group of 64 patients suspected of monoclonal gammapathy: 30 females (mean age 66yrs) and 34 males (mean age 64yrs). The subjects were treated in The Outpatient Clinic of Hematooncology and Marrow Transplantology and The Clinic of Hematooncology, SPSK-1 in Lublin.

Samples of blood were taken fasting, from the cubital vein to the test tubes without anticoagulants. Then the test

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tubes were centrifuged for 10min at 2,000rpm. Thus obtained serum was tested for free light chains of kappa and lambda type and by immunofixation to detect monoclonal protein. The testing was carried out in The Unit of Laboratory Diagnostics, SPSK-1 in Lublin.

Kappa and lambda free light chains were determined by FREELITE ™ kits (The Binding Site) on Simens BNII and Cobas Integra 400 analyzers.

Free light chains were determined by nephelometry on Siemens BN II. FREELITE ™ reagent kit is designed for quantitative analysis of FLCs. The method involves antigen-antibody reaction. The sample is treated with excess of specific antibody. Kappa and lambda FLCs bind to the specific antibody forming free light chain-specific antibody complexes. The beam of light passing through complex gets scattered. Intensity of scattered light measured by light detector set at angles other than 180° (usually 90° or 45°) is proportionate to FLC concentrations. Analytical sensitivity for kappa and lambda chains was 0.30 mg/L and 0.25 mg/L respectively.

Turbidimetric analysis of FLCs was done on Cobas Integra 400. The method involves measurements of the absorption of light passing through the examined sample. It is based on antigen-antibody reaction. FLCs concentration is proportionate to the intensity of light beam that passed through the sample/intensity of radiation beam. Analytical sensitivity for kappa and lambda chains was 0.6 mg/L and 1.3 mg/L.

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.

Immunofixation was done by Hydragel 4IF kit (Horiba ABX Diagnostics).

STATISTICAL ANALYSIS

The results were statistically analyzed by MedCalc and STATISTICA StatSoft 9.0.

Wilcoxon's test was used to determine significant differences between the results. Pearson's test assessed correlations between the results obtained by both methods. To verify diagnostic usefulness of the tested methods ROC curves and assessment of background fields were applied.

RESULTS

Table 1 presents results of statistical analysis of tested parameters depending on the method of kappa and lambda free light chain determination.Table1 presents the results of kappa and lambda FLCs determined simultaneously on both analyzers and evaluation of significant differences between the results of either method.

Moreover, we analyzed correlations between kappa and lambda FLC concentrations as well as obtained coefficients depending on the method applied.

Table 1. Descriptive statistics of variables

	Method and analyzer	XD ± SD	Me	Min.	Max.	Wilcoxon's test (p)
Kappa (mg/L)	nephelometry (Siemens BNII)	534.54 ± 1965.70	37.35	0.06	10900.00	<0.0001
Kappa (mg/L)	turbidimetry (Cobas Integra 400)	331.58 ± 1288.75	28.17	2.30	8950.20	
Lambda (mg/L)	nephelometry (Siemens BNII)	493.01± 1816.92	19.70	0.09	13300.00	0.8533**
Lambda (mg/L)	turbidimetry (Cobas Integra 400)	451.49 ± 1851.93	18.71	5.26	14130.57	
Kappa/ Lambda	nephelometry (Siemens BNII)	102.44 ± 333.86	1.17	0.00	1622.73	0.0004***
Kappa/ Lambda	turbidimetry (Cobas Integra 400)	50.77 ± 225.15	1.15	0.00	1564.72	

*p<0,001 **p=0,8533 ***p=0,0004
XD – mean, SD – standard deviation, Me-median, Min-minimum, Max-maksimum

The majority of correlations were statistically highly significant (p<0.01).

Figure 1 presents the results of kappa FLC concentrations obtained by either nephelometry (Siemens BNII) or turbidimetry (Cobas Integra 400) . The results of kappa FLC values obtained by either nephelometry (Siemens BNII) or turbidimetry (Cobas Integra 400) demonstrated highly significant correlation (R was positive, 0.9906) (Fig. 1).

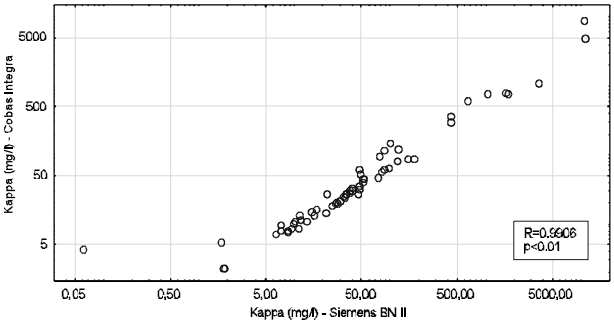

Fig. 1. Correlation between kappa FLCs determined by nephelometry and turbidimetry on logarithm scale

Figure 2 presents the results of lambda FLC concentrations obtained by either nephelometry (Siemens BNII) or turbidimetry (Cobas Integra 400). The results of lambda FLC values obtained by either nephelometry (Siemens BNII) or turbidimetry (Cobas Integra 400) demonstrated highly significant correlation (R was positive, 0.9821).

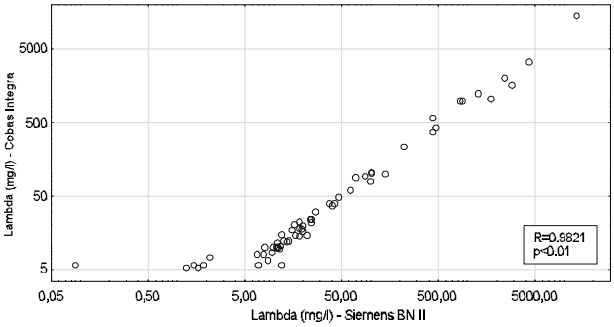

Fig. 2. Correlation between lambda FLCs determined by nephelometry and turbidimetry on logarithm scale

Figure 3 presents the values of kappa/lambda ratios calculated from the values of free light chains obtained by either nephelometry (Siemens BNII) or turbidimetry (Cobas Integra 400). The results demonstrated highly significant correlation (R was positive, 0.9860).

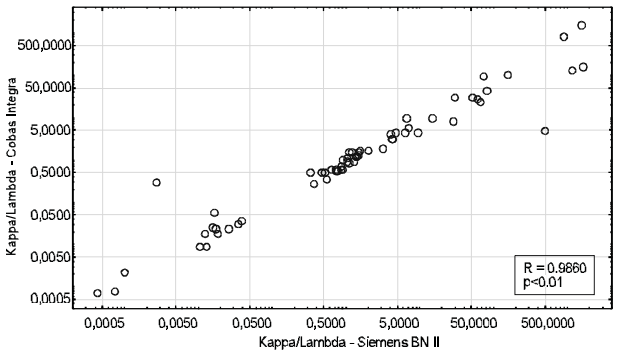

Fig. 3. Correlation between kappa/lambda FLC determined by nephelometry and turbidimetry on logarithm scale

To evaluate diagnostic value of kappa and lambda FLCs determined by various methods ROC curves were drawn depending on positive/negative immunofixation results (Fig. 4 and Fig. 5).

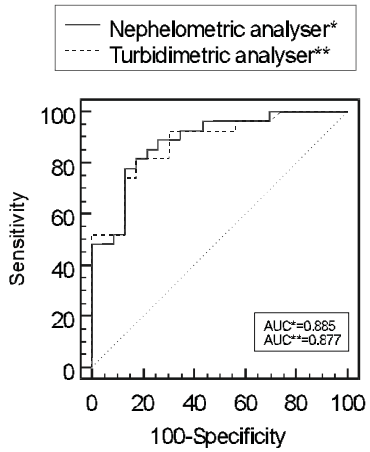

Fig. 4. ROC curves for kappa FLCs in patients with positive/negative immunofixation results (*Siemens BN II, **Cobas Integra 400)

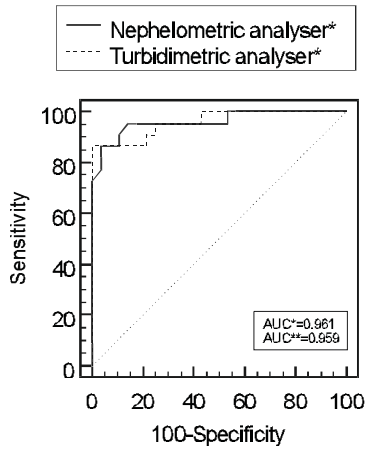

Fig. 5. ROC curves for lambda FLCs in patients with positive/negative immunofixation results (*Siemens BN II, **Cobas Integra 400)

Comparative analysis of ROC curves found bigger area under the ROC curve for lambda FLCs than kappa FLCs in both methods. For nephelometry on Siemens BNII analyzer the areas for kappa and lambda FLCs were 0.88 and 0.96 respectively; for turbidimetry on Cobas Integra 400 analyzer those areas were 0.87 and 0.95 respectively.

DISCUSSION

Electrophoresis and immunofixation of blood serum proteins are basic diagnostic methods used to detect and monitor monoclonal protein gammapathies. The methods allow to detect and identify the type of monoclonal protein. However the analyses of free light chains by nephelometry or turbidimetry have become common diagnostic tests recently. FLC analysis has become a valuable complementary method to the standard diagnosis of monoclonal gammapathies [4,7,9].

Many authors have advocated higher sensitivity of nephelometry and turbidimetry in detecting FLCs in comparison to protein electrophoresis and immunofixation. Immunofixation is able to detect FLCs at the concentrations of 100–150 mg/l at the least whereas nephelometry allowed their determination at the concentrations as little as 3–4 mg/l. It confirms the fact that the methods which use specific anti-kappa and anti-lambda antibodies allow to detect FLCs values within the reference range or below which is impossible with other accessible methods [2,4,8].

Our results confirm other research findings of high sensitivity of FLC determinations by nephelometry and turbidimetry. The lowest value of kappa FLC concentration obtained by nephlometry was 0.06 mg/L and by turbidimetry it was 2.30 mg/L. The concentrations of lambda FLCs were detected at a bit higher concentrations: 0.09 mg/L on nephelometry and 5.26 mg/L on turbidymetry.

Quantitative tests allow earlier detection of FLCs than standard immunofixation and electrophoresis. In patients with non-secretive myeloma and amyloidosis they are useful in monitoring patients with light chain disease especially [1,3,5].

Comparative analysis of both methods applied to detect kappa FLC in the group of patients examined found statistically highly significant differences ($p<0.0001$) between results depending on the method applied. The results of nephelometry on Siemens BN II are generally higher compared to turbidimetric values obtained on Cobas Integra 400. However determinations of lambda FLCs by those methods were not significantly different ($p=0.8533$). The results of lambda FLCs on nephelometry and turbidimetry were very close. Moreover, kappa/lambda ratios determined by nephelometry and turbidimetry were significantly highly different ($p=0.0004$) depending on the method. Kappa/lambda ratios determined by nephelometry were higher than the corresponding values on turbidimetry.

The figures present distribution of kappa and lambda FLC concentrations determined by nephelometry and turbidimetry which implies highly positive correlation between kappa and lambda FLCs (0.9906 and 0.9821 respectively). Additionally, high correlation was found between kappa/lambda ratios obtained by both methods. Statistical assessment of correlations confirms that both tests are equally useful in determining kappa and lambda chains.

Matters et al. [11] compared FLC values obtained on eight different analyzers. The examined 100 healthy persons who tested negatively on electrophoresis and 88 patients had with positive immunofixation results for myeloma and positive bone marrow biopsy results. They found that determinations of FLCs by nephelometric and turbidimetric analyzers were comparable and compatible.

The analysis of diagnostic usefulness of FLCs tested by both methods revealed considerable similarities. The area under ROC curve for kappa FLC on nephelometry (0.88) was slightly bigger than the area under the curve for turbidimetric method (0.87). Analogically, the area under ROC curve for lambda FLCs on nephelometry was a bit bigger than the area under ROC curve for turbidimetry. It is worth noting that the areas under the curves for lambda FLCs were bigger than the areas for kappa FLCs in both tests.

CONCLUSION

The analysis of results let conclude that nephelometric and turbidimetric techniques applied to determine kappa and lambda free light chains have comparable diagnostic value.

REFERENCES

1. Abraham R.S., Katzmann J.A., Clark R.J. et.al.: Quantitative analysis of serum free light chains. A new marker for the diagnostic evaluation of primary systemic amyloidosis. *Am. J. Clin. Pathol.,* 119, 274, 2003.
2. Bradwell A.R., Carr-Smith H.D., Mead G.P. et.al.: Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin. Chem.,* 47, 673, 2001.
3. Bradwell A.R., Carr-Smith H.D., Mead G.P. et.al.: Serum test for assessment of patients with Bence Jones myeloma. *Lancet* 361, 489, 2003.
4. Bradwell A.R.: *Serum Free Light Chain Analysis*. 6th ed. Bradwell 2010.
5. Drayson M., Tang L.X., Drew R. et. al.: Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. *Blood*, 97, 2900, 2001.
6. Jurczyszyn A., Skotnicki A.B.: *Szpiczak mnogi – kompleksowa diagnostyka i terapia*, Wgórnicki Wydawnictwo Medyczne, 2010.
7. Katzmann J.A., Abraham R.S., Dispenzieri A. et.al.: Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. *Clin. Chem.*, 51, 878, 2005.
8. Katzmann J.A., Clark R.J., Abraham R.S. et. al.: Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin. Chem.*, 48, 1437, 2002.
9. Kendziorek A., Bobilewicz D.M.: Badania laboratoryjne w różnych stadiach rozwoju gammapatii monoklonalnych. *In vitro Explorer,* 1, 3, 2007.
10. Levinson S.S., Keren D.F.: Free light chains of immunoglobulins: clinical laboratory analysis. *Clin. Chem.*, 40, 1869, 1994.
11. Matters D.J., Solanki M., Jones R. et.al.: Statistical analysis of free light chain results between eight nephelometric and turbidimetric platforms. *Hematology Meeting Reports,* 2, A5a, 2008.
12. Russo L.M., Bakris G.L., Comper W.D.: Renal handling of albumin: a critical review of basic concepts and perspective. *Am. J. Kidney Dis.*, 39, 899, 2002.
13. Usnarska-Zubkiewicz L., Hołojda J., Kuliczkowski K.: Serum free light chain (sFLC) – diagnostic and prognostic value in plasma cell dyscrasias. *Acta Haematol.* Pol., 40, 349, 2009.