ONCOLOGY

Serum Immunoglobulin Free Light Chains in Patients with Monoclonal Gammapathies

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Serum concentrations of κ- and λ-free light chains and their ratios were compared in 126 patients with monoclonal gammapathies (age 23-80 years) and 60 normal subjects (25-82 years). The main group included patients with intact immunoglobulin multiple myeloma, Bence Jones multiple myeloma, nonsecreting multiple myeloma, plasmocytoma, and monoclonal gammapathy of unknown origin. The accuracy of the method was evaluated by the analysis of 3 serum specimens with different concentrations of κ- and λ-free light chains. The variability of the method did not surpass the coefficient of variations permissible for this kind of analysis (10%). The new immunochemical method is characterized by high analytical sensitivity 100-fold surpassing that of electrophoretic methods. High concentrations of free light chains were most often found in the sera of patients with multiple myeloma with intact immunoglobulin secretion, Bence Jones multiple myeloma, and plasmocytoma. The diagnostic sensitivity of measurements of serum free light chains by the immunoturbidimetric method attained 90.5%. Combination of this method with serum protein electrophoresis and immunofixation resulted in detection of monoclonal gammapathy in 98.8% cases. These data indicate high specificity and analytical and diagnostic sensitivity of the immunoturbidimetric method for measurement of serum free light chains.

Key Words: *κ-free light chains; λ-free light chains; serum; monoclonal gammapathies*

Monoclonal plasmoproliferative diseases include a wide spectrum of abnormalities from benign monoclonal gammapathy of unknown origin (MGUO) to potentially curable solitary plasmocytoma and life-threatening multiple myeloma (MM) and light chain amyloidosis. Multiple myeloma is the most incident form of hematological malignancies, inferior by the incidence only to non-Hodgkin's lymphomas. Paraprotein secreted by MM malignant clone can be presented by molecules of intact immunoglobulin (MIIG), free light chains (FLC), or their combinations [8]. Plasma cells secrete five heavy chain isotypes and two FLC types, κ- and λ-FLC; the number of cells producing κ-FLC is 2-fold higher. In contrast to λ-FLC, which are dimers (50 kDa), κ-FLC molecules are monomers (25 kDa). Both FLC types can form highly polymerized forms. The production of light chains by normal and abnormal plasma cells is higher (up to 40%) than of heavy ones, this providing appropriate conformation of MIIG during their synthesis. FLC not included in MIIG are released into circulation and then filtered and metabolized in the kidneys depending on their molecular weight. κ-FLC pass through the glomerular filter much more rapidly than λ-FLC. The rate of

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glomerular filtration of polymers formed from FLC is slower than of κ- and λ-FLC molecules [10]. FLC circulating in the serum often form homodimers known as Bence Jones protein, marker of Bence Jones MM. The absence of paraprotein secretion is extremely rarely observed in nonsecreting MM.

The diagnosis, monitoring, and prognosis of monoclonal gammapathies were based on measurements of circulating monoclonal immunoglobulins. Analysis of serum and urinary proteins by electrophoresis and electrophoresis with immunofixation remained as the "golden standard" of screening for plasma cell diseases throughout a long time [6]. However, methods of FLC detection in the serum attracted much interest for several reasons. FLC are produced in all types of MM, including their production in the majority of patients with nonsecreting MM. In addition, measurements of FLC in daily urine do not reflect the rate of their synthesis by tumor cells, which is confirmed by the appearance of Bence Jones protein in the urine only in overflow proteinuria. The proximal renal tubules can metabolize FLC (up to 30 g/day), while its average production by plasma cells does not exceed 1 g/day [3]. Measurements of serum FLC directly reflect protein secretion by plasma cells and its level does not depend on the state of renal function. One of the main advantages of serum FLC assay is its use as an early marker of therapeutic response. This is explained by shorter half-life of FLC (several hours) in comparison with MIIG persisting in the circulation from 6 to 25 days [2]. Electrophoretic methods are semiquantitative and labor-consuming and their results depend on the accuracy of daily urine collection by the patient, qualification of the staff, and hence, cannot be reproduced in all laboratories. In addition, potentialities of these methods are limited in patients with small tumors or low production of FLC (oligosecreting MM) and patients with nonsecreting MM.

Initially the methods for serum FLC measurements were based on detection of the difference of the molecular weights of FLC and light chains in intact immunoglobulins. However, these methods were not widely used in clinical practice because of technological difficulties and insufficient accuracy. In early 2000s, a new automated immunoturbidimetric method for measurements of serum FLC has been developed. In contrast to the methods measuring the total concentration of light chains (FLC and light chains in immunoglobulins), the new method is based on the use of monoclonal antibodies highly specific for FLC [4]. This is attained by antibody reactions with the epitopes of light chains directly reacting with heavy chains during MIIG assembly. These epitopes are hidden in MIIG, while in the FLC molecule they are available for immunochemical reaction, due to which FLC can be detected with high precision in specimens with different concentrations thereof.

We evaluated analytical characteristics of immunoturbidimetric method for measurements of FLC and compared the serum concentrations of κ- and λ-FLC in patients with monoclonal gammapathies and healthy individuals (control group).

MATERIALS AND METHODS

A total of 128 patients (age 23-80 years) with monoclonal gammapathies were examined. The patients were hospitalized at Department for Therapy of Hematological Malignancies of B. B. Blokhin Center in June, 2008 – May, 2011 with the following diagnoses: 87 with MM with secretion of intact immunoglobulins (MMIIG), 18 with Bence Jones MM, 7 with nonsecreting MM, 6 with plasmocytoma, and 8 with MGUO. The diagnosis of plasma cell tumors was made in accordance with the international criteria for the diagnosis of monoclonal gammapathies, MM, and related diseases [9]. Control group consisted of 60 healthy men and women aged 25-82 years.

Serum concentrations of κ- and λ-FLC were measured by the immunoturbidimetric method on a Hitachi 911 automated biochemical analyzer using Freelite Human Lambda and Freelite Human Kappa kits (Binding Site). The κ/λ ratio was calculated. The results beyond the technological potentialities of the method were obtained by multiple successive dilutions in accordance with the protocols.

The data were statistically processed by Kruskal–Wallis method. Correlations were evaluated by Spearman's nonparametric test. The threshold values were calculated on the base of ROC analysis and 95% confidence interval. The differences of incidence in the groups were evaluated by nonparametric χ^2 test. The differences were considered significant at $p<0.05$.

RESULTS

The advantage of the immunoturbidimetric method for FLC measurements is its high analytical sensitivity: the threshold level for κ-FLC detection is 1.5 mg/liter, for λ-FLC 3 mg/liter, which is 10-fold higher than the threshold levels detectable by electrophoresis in daily urine (10-20 mg/liter) and by several orders of magnitude higher than the threshold levels detected by serum electrophoresis (500-2000 mg/liter) and serum electrophoresis with subsequent immunofixation (150-500 mg/liter, Table 1). Our findings suggest that the coefficient of variations increases in a series of measurements and between different series from specimens with lower concentration to specimens with higher FLC concentration. The variability of κ-FLC

measurements in a series (3.0-7.8%) was higher than for λ-FLC (2.6-4.9%), while κ-FLC variability between different series was lower (4.5-7.6%) than for λ-FLC (6.3-9.8%). Hence, variability of the method did not surpass the coefficient of variations permissible for analysis of this kind (10%).

Measurements of serum FLC by the immunoturbidimetric method in normal subjects showed that the κ- and λ-FLC concentrations were comparable with the reference values recommended by kit manufacturer (κ-FLC: 3.3-19.4; λ-FLC: 5.7-26.3; κ/λ: 0.25-1.65; Table 2). Despite the 2-fold higher production of κ-FLC, the serum concentration of λ-FLC was somewhat higher (5.7-22.0 mg/liter). This contradiction can be explained by a higher rate of glomerular filtration of κ-FLC, which are monomers, while λ-FLC molecules are dimers. Analysis of the results of FLC measurements in the control revealed a direct highly significant linear relationship between κ- and λ-FLC (r_s=0.782, p<0.0001). On the other hand, the correlation was inverse in patients with MMIIG and Bence Jones MM and also highly significant (r_s=-0.55 and r_3=-0.6, respectively; p<0.05), indicating disorders in FLC synthesis and secretion by plasma cells in these patients. No relationship between serum FLC levels and gender or age was detected in normal subjects.

The greatest variability of κ-FLC, λ-FLC, and κ/λ has been found in patients with MMIIG and Bence Jones MM. This can be due to the fact that malignant plasma cells most often secrete the same FLC type (the so-called FLC involved in the pathological process). As a result, the concentrations of FLC involved in the pathological process in the circulatory bed can surpass the normal values hundreds and thousand times. On the other hand, secretion of FLC not involved in the pathological process (λ-FLC for κ-type myeloma and κ-FLC for λ-type myeloma) can remain unchanged. However, the patients most often develop bone marrow suppression of the alternative FLC synthesis and secretion by normal plasma cells. Hence, the κ/λ ratio can reach extremely high or low values, depending on the MM secretion type. The level of κ-FLC in patients with MMIIG and Bence Jones MM differed significantly from that in the control group (p=0.001 and p=0.02, respectively). Highly significant differences in κ-FLC concentrations were observed also in patients with MMIIG (p<0.02) and Bence Jones MM (p<0.05), in contrast to the groups with nonsecreting MM, plasmocytomas, and MGUO. Serum levels of FLC involved in the pathological process in these patients surpassed the normal level by one order of magnitude and reached 19,699 mg/liter in MMIIG and 9638 mg/liter in Bence Jones MM.

The concentrations of λ-FLC in patients with nonsecreting MM were close to the reference values, except one patient with κ-FLC concentration 676 mg/liter and κ/λ=62, which several hundred times surpassed the corresponding upper reference values, while the results of electrophoresis were negative. Only λ-FLC values differed significantly (p<0.05) from the control group in MGUO patients. In plasmocytoma patients the studied parameters virtually did not differ from the control, which could be explained by small size of the group.

In order to evaluate the diagnostic value of the studied FLC, their threshold values were calculated from the data in the control group. In accordance with the standard requirements to statistical analysis, the threshold values were calculated with consideration for the mean value and two standard deviations, which corresponded to 95% confidence reference interval. The threshold value for κ-FLC was 21.5 mg/liter, for κ-FLC 27.0 mg/liter. Elevation of κ-FLC concentration was found most often: in 72% patients with Bence Jones MM (13 of 18 cases), 66% (57 of 87 cases) in MMIIG, and 50% in plasmocytoma (3 of 6 cases), while enhanced λ-FLC secretion was found in 28, 23, and 17% cases, respectively. In MGUO group, patients

TABLE 1. Variability of Measured Levels of Serum κ-FLC and λ-FLC

Specimen	Intraseries variability				Variability between series			
	κ-chains		λ-chains		κ-chains		λ-chains	
	MV, mg/liter	KV, %	MV, mg/liter	KV, %	MV, mg/liter	KV, %	MV, mg/liter	KV, %
1	8.0	7.8	11.8	4.9	8.4	7.6	12.5	9.8
2	27.2	7.3	24.6	4.6	27.8	7.2	25.3	7.8
3	46.9	3.0	40.4	2.6	41.7	4.5	38.5	6.3

Note. MV: mean value; KV: coefficient of variations.

TABLE 2. Concentrations of κ- and λ-FLC and Their Ratio in Patients with Monoclonal Gammapathies and in Healthy Individuals

Group	Concentration, mg/liter		
	κ-FLC	λ-FLC	κ/λ
Control	14.7 (7.3-20.8)	12.1 (5.7-22.0)	1.25 (0.75-1.64)
MM with intact immunoglobulin secretion	126 (3.7-14 950)	9.8 (1.2-19 699)	14.7 (0.0008-2631)
Bence Jones MM	251 (6.4-9638)	9.7 (2.0-3932)	28.9 (0.003-1365)
Nonsecreting MM	13.6 (11.7-676)	7.7 (6.3-17.0)	1.86 (0.96-62.6)
Plasmocytoma	19.9 (13.4-148)	12.2 (7.9-61)	1.69 (0.23-15.2)
MGUO	16.6 (10.4-190)	25.5 (8.3-137)	0.57 (0.08-18.1)

Note. Results are presented as medians and minimum and maximum values.

with high levels of λ-FLC predominated (50%) and patients with high κ-FLC level constituted 38%. The secretion of two FLC isotypes remained normal in 33% patients (2 of 6) with plasmocytoma, in 12.5% patients (1 of 8) with MGUO, and in 13.8% patients (12 of 87) with MMIIG. The fact that FLC concentrations were normal in 12 patients with MMIIG indicated secretion of MIIG alone. This was confirmed by electrophoresis of serum and urinary proteins. Serum levels of κ- and λ-FLC were elevated in 2 patients with MMIIG, which could be caused by pronounced renal failure by the moment of examination.

Of 57 patients with MMIIG with high serum κ-FLC levels, secretion of λ-FLC was normal in 42 patients and low in 13, which attests to suppressed synthesis and production of λ-FLC by plasma cells. On the other hand, high level of κ-FLC was associated with normal level of λ-FLC in 10 of 13 patients with Bence Jones MM (77%), while in 3 patients (23%) serum λ-FLC levels were low. No patients with serum κ-FLC level below the threshold value (3 mg/liter) were detected in the examined cohort. On the other hand, λ-FLC level was low in comparison with the corresponding threshold value (5 mg/liter) in 15% (13 of 87) patients with MMIIG and in 17% (3 of 18) patients with Bence Jones MM. The data on the incidence of high FLC levels in the patients are in line with the concepts according to which the B-lymphocytopoiesis process starts from restructuring of the gene sites responsible for the κ-FLC molecule construction, this creating the quantitative predomination of plasma cells producing κ-FLC [1].

According to various authors, the κ/λ ratio, in contrast to electrophoretic parameters, is a quantitative indicator of clonality and a more sensitive marker of monoclonal secretion than elevation of FLC level [7]. The significance of this parameter increases in κ-FLC secretion and decreases in λ-FLC secretion, but remains unchanged in production of polyclonal immunoglobulins and/or renal dysfunction, when the concentrations of both FLC types may increase 30-40-fold. This was confirmed by our data. The κ/λ ratio in the MMIIG group was elevated in 63% cases, low in 21%, and normal in 16% cases. It was high in the overwhelming majority (72%) of patients with Bence Jones MM and low in 28%, hence, was abnormal in all patients. The κ/λ ratio was high in the majority of patients with nonsecreting MM (57%) and plasmocytoma (67%). The κ/λ ratio in patients with MMIIG and Bence Jones MM (14.7 and 28.9, respectively) was significantly higher than in the control group ($p<0.001$), as well as in patients with nonsecreting MM, plasmocytoma, and MGUO ($p<0.05$). The κ/λ ratio virtually did not differ in the groups of patients with MMIIG and Bence Jones MM.

The diagnostic sensitivity of the method for measurements of serum FLC was compared with that of standard electrophoretic methods for analysis of serum and urinary paraprotein (Fig. 1). Electrophoresis with subsequent immunofixation of serum proteins detected paraprotein in 88.1% primary patients, while electrophoresis with subsequent immunofixation of urinary proteins detected in only 66.7% cases; all electrophoretic methods detected the target protein in 96.4% cases. The immunoturbidimetric method detected monoclonal secretion in 90.5% patients. Combined use of the κ/λ ratio and electrophoresis of the serum and urine increased the diagnostic sensitivity to 98.8%. Combination of the κ/λ ratio and electrophoresis of the serum with subsequent immunofixation gave the same result. These data indicate that urinary protein electrophoresis as an additional diagnostic procedure in patients with suspected monoclonal gammapathy has little diagnostic value.

According to published data, the threshold concentration of FLC causing overflow proteinuria and appearance of Bence Jones protein in the urine is

113 mg/liter for κ-FLC and 278 mg/liter for λ-FLC [9]. High serum levels of κ-FLC (26.9-945.0, median 237.0 mg/liter) were found in primary patients with trace amounts of Bence Jones protein in the urine. Importantly that serum κ-FLC was detected in comparable high concentrations (24.4-884.0, median 126.5 mg/liter) in the patients without urinary FLC detectable by the standard methods. Hence, the range of serum κ-FLC concentrations in the patients with negative (no Bence Jones protein) and positive results of daily urine electrophoresis virtually coincided. This indicated a higher diagnostic and analytical sensitivity of the immunoturbidimetric measurements of FLC in comparison with electrophoretic analysis. One of the reasons for which FLC could not be detected in the urine sometimes was polymerization of FLC molecules (largely κ-FLC), particularly when in high concentrations, with the formation of high-polymeric forms. FLC polymers passed through the glomerular barrier much slower, which impedes their detection in the urine. In addition, their electrophoretic track could be blurred, looking like a polyclonal background, detection of this track precluding its characterization as a monoclonal fraction.

Immunoturbidimetric measurement of FLC can be also used for monitoring of the treatment of patients with monoclonal gammapathies. Due to high analytical sensitivity of the method, it is particularly important for patients with nonsecreting MM, oligosecreting MM, and Bence Jones MM. The concentrations of FLC involved in the pathological process decreased significantly in effectively treated patients with Bence Jones MM (medians before and after therapy 1133 and 35 mg/liter, ranges 364-2743 and 14-174 mg/liter, respectively). Excretion of Bence Jones protein in screening was 0.19-2.05 g/day with subsequent reduction to trace levels or complete absence. On the other hand, serum levels of FLC involved in the pathological process increased again from 13.4 (8.6-231) to 304.0 (38.2-4257) mg/liter, as did serum paraprotein level and urinary level of Bence Jones protein in MMIIG patients in whom complete or partial remission was attained after disease progress. In patients treated ineffectively, all the parameters remained at virtually the initial level.

Studies of FLC involve certain difficulties of their detection. In some cases, testing of successive dilutions of patients' sera showed high variability of FLC concentrations, with the values differing 10-100-fold. Disorders of linearity in analysis could be caused by excessive amount of the antigen, when insufficient level of antibodies could lead to erroneously low result. The FLC exist in the forms of monomers or dimers, but can form highly polymerized forms. In immunoprecipitation reaction these form act as a multi-antigen, which can show erroneously high concentrations

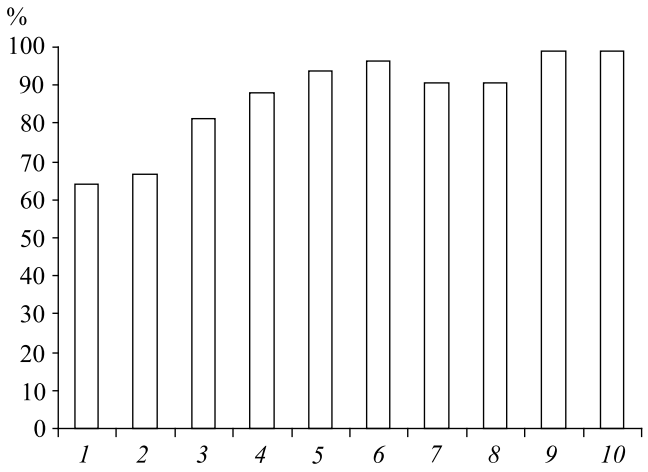

Fig. 1. Diagnostic sensitivity of methods for paraprotein measurements in MM. *1*) electrophoresis of urinary proteins (EPU); *2*) EPU+immunofixation (IF); *3*) electrophoresis of serum proteins (EPS); *4*) EPS+IF; *5*) EPS+EPU; *6*) EPS+EPU+IF; *7*) FLC involved in pathological process; *8*) κ/λ; *9*) κ/λ+EPS+IF; *10*) κ/λ+EPS+EPU+IF.

of FLC. The antigen-antibody reaction can be also disordered by point mutations and modified conformation of the FLC protein molecule, as a result of which the antibodies would not react with this antigen and the result of FLC measurement would be too low [5,11]. However, automated and manual dilutions intended in the protocols of the method and serial studies of specimens reduce the risk of unreliable results.

Our data demonstrate high specificity and sensitivity of the method for measurements of serum FLC. The use of the automated method and serum as an object of analysis promote more accurate and reproducible results. Measurement of serum FLC has good prospects for wide medical use. Addition of serum FLC measurements to protocols of examinations of patients with suspected monoclonal gammapathy will improve the diagnostic sensitivity of the available methods for paraprotein measurements and will make it possible to monitor patients with nonsecreting MM and to exclude analysis of daily urine. Short half-life of FLC molecules in comparison with intact immunoglobulin will promote monitoring with maximally rapid evaluation of response to therapy, its efficiency, and presence of residual disease.

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