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# Immunochemical Diagnosis of Multiple Myeloma

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The diagnostic potentialities of complex immunochemical analysis of the serum and daily urine were evaluated in 118 patients with multiple myeloma. In 95 patients, we observed secretion of monoclonal intact immunoglobulins with heavy chains G (*N*=69), A (*N*=19), and M (*N*=4) and biclonal secretion of paraproteins G and A (*N*=3). Bence-Jones protein was detected in the sera and daily urine of 16 patients and Bence-Jones proteinuria alone was detected in 3 patients. The diagnostic sensitivity of serum immunoelectrophoresis in multiple myeloma is 94.1%. Analysis of paraproteinuria is particularly important in Bence-Jones myeloma, when paraprotein excretion may be not associated with paraproteinemia. Complex study by immunoelectrophoretic and immunoturbidimetric methods in multiple myeloma increases the diagnostic sensitivity to 99.2%.

**Key Words:** *multiple myeloma; monoclonal secretion; paraproteins; Bence-Jones protein; diagnosis*

Monoclonal immunoglobulins reflecting pathological proliferation of plasma cells have been described as the first serological tumor marker [4,11]. Modern diagnosis of multiple myeloma (MM) includes complex study of the blood and urinary abnormal proteins characterizing monoclonal secretion of the pathological clone of the plasma cells [7,10].

Paraproteins are monoclonal immunoglobulins or their fragments, products of the same clone of plasma cells or B lymphocytes. Paraprotein can be presented by molecules of intact immunoglobulin, free light chains (FLC) of immunoglobulins, and by their combinations. Plasma cells produce 5 isotypes of heavy chains (G, M, A, D, and E) and two FLC types — κ- and λ-FLC [1]. The unique structure of paraproteins makes them specific for each clone of plasma cells [11].

Paraproteins are detected by the method of electrophoresis of the serum and daily urine proteins by the presence of M-gradient, usually in the γ-globulin zone. In some cases, there may be more than one band due to dimerization or presence of paraprotein complexes or fragments. Paraproteinemia is identified by immunofixation with the use of specific antisera to the immunoglobulin main heavy and light chains [4,8,11].

Normal and abnormal plasma cells produce the light chains in higher amounts (up to 40%) than heavy ones, which ensure appropriate conformation of monoclonal immunoglobulins during their synthesis. FLC circulating in the serum often form homodimers known as Bence-Jones protein, a marker of Bence-Jones myeloma. The potentialities of the diagnosis of paraproteinemias were improved with the development of immunoturbidimetric detection of serum FLC [2,3,5,6].

Here we evaluate diagnostic potentialities of a complex immunochemical analysis of the serum and 24-h urine in patients with MM.

## MATERIALS AND METHODS

The study included 118 patients (60 women and 58 men aged 25-82 years) with the diagnosis of MM, who were examined and treated at N. N. Blokhin National Research Medical Center of Oncology in 2013-2016. The disease was diagnosed in accordance with the international criteria for MM diagnosis [10]. The pa-

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tients received no specific therapy before examination. The control group consisted of 98 age-matched healthy men and women.

Paraproteinemia and Bence-Jones proteinemia were diagnosed by immunofixation electrophoresis (Hydrasys, Sebia) with the use of Hydragel 2/4 IF, Hydragel 2/4 Bence-Jones test systems and specific antisera to the main types of immunoglobulin heavy and light chains. The concentrations of immunoglobulins (IgG, IgA, and IgM), total protein, albumin, β2-microglobulin (β2-MG) were measured on a Cobas 6000 automated analyzer (Roche). Secretion of κ- and λ-FLC (mg/liter) was measured in the serum by the immunoturbidimetric method on an Advia 1800 automated biochemical analyzer with the use of Freelite Human Lambda and Freelite Human Kappa test systems (Binding Site). The blood was collected from the ulnar vein and centrifuged for 10 min at 3000 rpm. Analysis of proteinuria was carried out in 24-h urine. Serum and urine samples were stored at 4°C no longer than 7 days.

The results were statistically processed using Statistica 7.0 software (StatSoft, Inc.) by nonparametric Mann—Whitney test. The data were presented as the median with intervals. The differences were considered significant at $p<0.05$.

## RESULTS

Immunoelectrophoresis of the sera detected signs of monoclonal protein secretion in 111 patients. No electrophoretic signs of monoclonal secretion in the blood or urine were found in 4 patients, which corresponded to nonsecretory myeloma.

Secretion of monoclonal intact immunoglobulins with heavy chains G (*N*=69), A (*N*=19), and M (*N*=4) and biclonal secretion of paraproteins G and A (*N*=3) was identified in 95 patients with MM. Bence-Jones protein was detected in the serum and daily urine of 16 patients, in 3 patients it was detected only in daily urine.

Hence, the patients were distributed into the following groups: G myeloma (58.5%), A myeloma (16.1%), Bence-Jones myeloma (16.1%), M myeloma (3.4%), myeloma with biclonal secretion of paraproteins G and A (2.5%), and nonsecretory myeloma (3.4%). Immunochemical and biochemical parameters were analyzed in all groups (Table 1).

**TABLE 1.** Serum Immunochemistry and Biochemistry in MM

| Parameter | G- myeloma (*N*=69) | A- myeloma (*N*=19) | M- myeloma (*N*=4) | Biclonal G+A myeloma (*N*=3) | Bence-Jones myeloma (*N*=19) | Nonsecretory myeloma (*N*=4) | Reference intervals |
|---|---|---|---|---|---|---|---|
| Paraproteinemia, g/liter | 28.6 (1.1-95.9) | 24.3 (3.7-73.5) | 24.7 (61.2-52.3) | 78.8 (8.9-83.9) | 2.64 (0.13-38.2) | — | — |
| Total protein, g/liter | 91.1 (65.4-147.6) | 84.1 (63.5-118) | 87.4 (69.1-102.0) | 137.6 (68.5-146.2) | 68.5 (61.0-96.3) | 72.6 (66.9-81.6) | 66-87 |
| Albumin, g/liter | 38.9 (20.5-50.0) | 40.0 (23.5-48.4) | 34.9 (32.1-42.2) | 37.6 (36.8-40.2) | 45.4 (35.4-65.9) | 44.8 (44.6-48.5) | 35-50 |
| β2-MG, mg/liter | 3.41 (1.4-20.6) | 4.47 (1.7-21.8) | 7.2 (1.1-3.9) | 4.51 (3.3-6.2) | 4.23 (1.79-26.3) | 1.86 (1.34-3.9) | 0.8-2.4 |
| IgG, g/liter | 37.5 (5.7-129.1) | 4.96 (2.6-7.3) | 9.44 (5.8-13.4) | 99.1 (6.31-110.60) | 5.9 (3.4-15.0) | 7.8 (6.8-13.0) | 7-16 |
| IgA, g/liter | 0.51 (0.08-6.20) | 26.7 (4.0-60.8) | 1.06 (0.58-3.20) | 3.89 (0.28-6.30) | 0.57 (0.13-2.50) | 0.57 (0.81-3.40) | 0.7-4.0 |
| IgM, g/liter | 0.26 (0.05-5.50) | 0.26 (0.09-5.20) | 41.1 (2.2-77.1) | 0.28 (0.20-0.53) | 0.22 (0.07-1.30) | 0.59 (0.3-1.5) | 0.4-2.3 |
| κ-FLC, mg/liter | 241.8* (0.47-102,640.00) | 25.6* (10.7-257.0) | 1557* (1210-64,500) | 47.6* and 768.9* | 4358* (26.3-39,480.0) | 17.9 (4.6-82.4) | 3.0-21.5 |
| λ-FLC, mg/liter | 374.2* (0.39-12,430.00) | 195.2* (16.9-12,936.0) | 40.8* | 273.1* | 3418* (36.7-20,375.0) | 18.1 (7.9-91.2) | 5-27 |

**Note.** *Secretion of κ- or λ-FLC was estimated for groups with the respective involved FLC.

Analysis of the types of involved immunoglobulin light chains detected secretion of κ-light chain paraproteins in 69 patients, λ-light chain paraproteins in 42, and biclonal secretion of κ- and λ-FLC in 3 patients.

Electrophoretic mobility of paraprotein G was detected mainly in the γ-globulin zone (92.7%). Paraprotein A was more often detected in the β-globulin zone (84.2%) and rarely in the γ- and α2-globulin zone. In three cases, the secretion of paraprotein M coincided with the γ-globulin zone and in just one case — with the α2- and β-globulin zone. Bence-Jones paraproteinemia was detected in the γ-globulin zone in 62.5% cases.

According to immunoelectrophoresis, paraproteinemia in the group was in general characterized by manifest variability: from 0.13 to 95.9 g/liter (median 22.1 g/liter).

The maximum secretion of monoclonal protein was detected in the G myeloma group: 95.9 g/liter (Gκ paraproteinemia), with the median of 28.6 g/liter. Serum hyperproteinemia reaching 147 g/liter (median 91.1 g/liter) corresponded to manifest paraproteinemia. Albumin median in this group of patients was 39.9 g/liter, at β2-MG median of 3.41 mg/liter. Secretion of IgG reached the peak — 129.1 g/liter (median 37.5 g/liter), which significantly surpassed the normal (7-16 g/liter).

The maximum secretion of paraprotein in patients with A myeloma was 73.5 g/liter (median 24.3 g/liter). In addition, this group was characterized by hyperproteinemia with the maximum concentration of total protein 118 g/liter (median 84.1 g/liter). The medians of albumin (40.0 g/liter) and β2-MG (4.5 mg/liter) did not differ from the corresponding values in G myeloma. The IgA median in patients with A myeloma was 26.7 g/liter (max 60.8 g/liter), which significantly surpassed the normal (0.7-4.0 g/liter).

The levels of paraprotein in patients with M myeloma varied from 1.15 to 52.3 g/liter (median 24.8 g/liter). The median of total serum protein was 84.4 g/liter, albumin median 34.9 g/liter, and β2-MG median 7.2 mg/liter. Secretion of IgM in this group of patients reached 77.1 g/liter (median 41.1 g/liter), which surpassed the normal (0.4-2.3 g/liter).

Paraproteinemia median in patients with Bence-Jones myeloma characterized by FLC production was significantly ($p<0.00005$) lower than in MM with secretion of intact immunoglobulins (G, A, and M myeloma). The median of paraprotein secretion in the group of patients with Bence-Jones myeloma was 2.6 (0.13-38.1) g/liter. Total protein level in Bence-Jones myeloma (68.5 g/liter) was also significantly ($p<0.001$) lower than in MM with the production of intact immunoglobulins. On the other hand, the medians of albumin (45.4 g/liter) and β2-MG (4.2 mg/liter) in Bence-Jones myeloma in fact did not differ from the corresponding values in other MM types. The median serum concentrations of IgG (5.9 g/liter), IgA (0.58 g/liter), and IgM (0.22 g/liter) in Bence-Jones myeloma were lower than the reference levels.

Secretion of each FLC type was analyzed in patients with the respective monoclonal FLC, also called "involved FLC" [3,10]. The type of monoclonal light chains was verified by immunoelectrophoresis.

The highest median of κ-FLC secretion was detected in patients with Bence-Jones myeloma (4358 mg/liter), the maximum value reaching 39,480 mg/liter. The median of κ-FLC secretion was lower in patients with G myeloma (241.8 mg/liter), though its maximum secretion reached 102,640 mg/liter. Manifest hypersecretion of κ-FLC was recorded also in M myeloma (maximum 64,500 mg/liter), while in A myeloma the secretion of κ-FLC was lower than in other MM types (median 25.6; 10.7-256.7 mg/liter).

Secretion of λ-FLC was characterized by the maximum median in patients with Bence-Jones myeloma — 3417 (36.7-20,375.0) mg/liter. The levels of λ-FLC hypersecretion in G myeloma (374.2; 0.39-12,430.00 mg/liter) and A myeloma (195.2; 16.9-12,936.0 mg/liter) were lower than in Bence-Jones myeloma. Secretion medians for κ-FLC and λ-FLC in patients with nonsecretory myeloma did not differ from the control; however, high secretion of κ-FLC was detected in 2 cases (22.4 and 82.4 mg/liter) and of λ-FLC in one case (91.2 mg/liter), without electrophoretic signs of monoclonal secretion. Hence, immunoturbidimetric analysis of FLC was effective in 75% cases with nonsecretory myeloma, when monoclonal secretion was not detected by immunoelectrophoretic methods.

Analysis of the diagnostic sensitivity of immunoelectrophoresis of the serum in the total group of MM patients demonstrated that monoclonal secretion could be detected in 94.1% cases. In complex with the immunoturbidimetric analysis of FLC, the efficiency of detection of monoclonal secretion reached 99.2%, which was in line with the previous data [2,9,11].

In addition to the serum, specimens of 24-h urine were analyzed in MM patients. Urinary total protein median was 0.36 g/day in the entire group of patients with MM, the maximum values were recorded in Bence-Jones myeloma (19.1 g/day). Immunoelectrophoresis of daily urine detected Bence-Jones paraproteinuria in 39.1% of cases with G myeloma and 21.1% cases with A myeloma. Paraproteinuria was detected by immunoelectrophoresis of the urine in all patients with Bence-Jones myeloma. Bence-Jones urinary protein median was the highest in Bence-Jones myeloma (1.29 g/day), its maximum level reaching 12.1 g/day.

Bence-Jones proteinuria was significantly lower in G myeloma (0.32 g/day; $p$=0.03) than in Bence-Jones myeloma. Our data indicate that the diagnosis of paraproteinuria is particularly important in Bence-Jones myeloma, characterized in many cases by urinary excretion of monoclonal proteins in the absence of paraproteinemia (15.8% of all Bence-Jones MM).

Complex immunochemical analysis with the use of electrophoretic and immunoturbidimetric methods demonstrated their high efficiency for the diagnosis of various MM types. Electrophoretic study identified the paraprotein subtype; the method was more sensitive for MM with secretion of intact immunoglobulins. Immunoturbidimetric method for studies of FLC was more sensitive for detection of γ-pathies with light chain secretion (for example, Bence-Jones MM) and for cases without paraprotein secretion detectable by electrophoresis. Combined use of electrophoretic methods and immunoturbidimetry improved the diagnostic sensitivity of immunochemical studies in MM, which reached 99.2%.

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