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EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Free Immunoglobulin Light Chains as Criteria of Extracorporeal Hemocorrection in Patients with Monoclonal Gammopathies

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Elimination of free immunoglobulin light chains with the use of EMic2 selective filters was carried out in 12 patients with monoclonal gammopathies and high production of free immunoglobulin light chains. We showed that extracorporeal detoxification for direct removal of excessive free immunoglobulin light chains from the circulation is advisable for these patients, irrespective of the presence and severity of renal insufficiency. Rapid reduction or elimination of free light chains of immunoglobulins in the course of selective extracorporeal elimination helps to prevent the development of irreversible renal failure and to perform adequate antitumor therapy.

Key Words: *immunoglobulins free light chains; monoclonal gammopathy; multiple myeloma; lymphoma; extracorporeal hemocorrection*

Renal failure often aggravates the course of monoclonal gammopathies. Renal failure is the first and the main clinical manifestation of multiple myeloma (MM) in 18-56% patients, 12-20% patients present with acute renal failure (ARF), and 10% patients are in need of program hemodialysis [6,7]. The presence and severity of renal failure often determine the potentialities of adequate antitumor therapy and prognosis. Renal function recovery is associated with better survival [10].

Immunoglobulins free light chains (FLC) have low molecular weight (23,000-46,000 Da) and, in contrast to heavy chains, normally cross the glomerular filter and are reabsorbed by the proximal tubular epithelium, which maintains their stable blood concentration (22 and 27 mg/liter for κ- and λ-FLC, respectively). Proliferation of monoclonal plasma cells in MM can lead to a drastic increase (by several thousand times) of serum FLC concentrations. The main cause of renal damage in MM is cylinder nephropathy caused by precipitation of FLC with Tamm—Horsfall protein in the distal parts of the renal tubules inducing interstitial inflammation and obstructive ARF [5]. Plasma exchange technologies have been used for many years for direct extracorporeal elimination of excessive FLC from systemic circulation; the results indicated the possibility of 50% reduction of FLC concentration [8,11]. However, the efficiency of plasma exchange in various methodological approaches (number of plasma exchange operations, FLC level/exfused plasma volume proportion, *etc.*) proved to be less than expected as regards the degree of reduction of serum FLC concentration [3]. This fact can be attributed to large volume of FLC distribution in other liquid media of the

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body and in tissues, with the intravascular FLC content of 15-20% of their total content. Removal of 3.5 liters of plasma in the course of each plasma exchange procedure is associated with elimination of no more than 65% intravascular FLC; intensification of plasma exchange leads to inevitable loss of plasma clotting factors, hypoproteinemia, and hypoalbuminemia with subsequent threatening secondary complications.

An alternative method of extracorporeal elimination of FLC is now used more and more often: hemodialysis with the use of membranes that eliminate the substances with threshold molecular weight of up to 50,000 Da without secondary complications associated with deproteinization.

We evaluate the efficiency of elimination of excessive FLC with the use of EMic2 selective membranes (Fresenius) in the course of hemoperfusion by measuring the concentrations of κ- and λ-FLC in the serum and dialysate in patients with monoclonal gammopathies before and during therapy.

MATERIALS AND METHODS

Extracorporeal hemocorrection was carried out in 12 patients: 9 with MM, 2 with diffuse B-large-cell lymphoma, and 1 with Waldenström disease. In accordance with the common criteria, FLC levels above 500 mg/liter irrespective of azotemia level served as an indication for starting the procedure [9].

Extracorporeal detoxification was carried out on Fresenius hemodialysis machine. The stationary (4008) or mobile (Multifiltrat) variants were used, depending on the need of FLC level monitoring in dialysate. Polysulfone filter EMic2 (Fresenius) with the membrane area of 1.8 m^2, filtering and removing substances with molecular weight of up to 60,000 Da, were used. The vascular access was created by a perfusion catheter, inserted into the femoral or subclavian vein. Blood flow velocity during hemodialysis varied from 200 to 250 ml/min, the duration of each procedure 4-6 h. Regulated clotting by nonfractionated heparin was calculated individually from coagulogram values. During extracorporeal detoxification, all patients received standard antitumor therapy for prevention of filtration loss and maintenance of the due concentrations of drugs, which were administered after hemodialysis procedure was over. No early and delayed complications of extracorporeal hemocorrection were recorded.

The treatment of lymphoproliferative diseases was carried out in accordance with the common standards of hospital care without modification of protocols or drug dose reduction.

Serum concentrations of κ- and λ-FLC (mg/liter) were measured by the immunoturbidimetric method on Advia 1800 automated biochemical analyzer using Freelite Human Lambda and Freelite Human Kappa test systems (Binding Site). The results beyond the technological threshold of the method were obtained by many-fold successive dilutions in accordance with the programs. The weights of eliminated FLC were calculated with consideration for the individual circulating blood volumes (CBV). Paraproteinemia was diagnosed by immunofixation electrophoresis (Hydrasys; Sebia) with specific antisera to the main types of immunoglobulin heavy and light chains.

The results were statistically processed using Statistica 7.0 software (StatSoft) using Mann—Whitney nonparametric test. The differences were considered significant at $p<0.05$.

RESULTS

This paper presents the first experience gained in FLC elimination by hemodialysis with EMic2 selective membranes in 12 patients with paraproteinemic hematological malignancies with consideration for the results of immunochemical analysis of the serum. Electrophoretic studies with immunofixation showed paraproteinemia of various types in all patients: Gκ paraproteinemia in 2 patients, Gλ in 4, Mκ in 2, and one case of each of the following variants: κ type Bence Jones paraproteinemia, Gκ and Aκ biclonal secretion, and Mκ paraproteinemia combined with type κ Bence Jones paraproteinemia. Paraproteinemia level at primary examination varied from 1.6 to 78.8 g/liter.

Six patients exhibited a manifest increase in κ-FLC secretion and 6 others of λ-FLC, which were denoted as involved chains. An FLC secreted by a pathological cell clone was assumed to be an involved FLC, which was confirmed by immunofixation electrophoretic study of the serum and daily urine. The primary levels of involved FLC were 212-74,900 mg/liter for κ-FLC and 575-34,784 mg/liter for λ-FLC. The highest levels of κ- and λ-FLC were detected in MM. Hypersecretion of κ-FLC (212 and 1205 mg/liter) was detected in patients with non- Hodgkin's lymphomas. All patients exhibited proteinuria of 0.16 to 18.7 g/day, Bence Jones protein excretion (from 0.04 to 14.2 g/day) was detected in 9 cases.

Ten patients presented with renal failure with creatinine level of 263-764 μmol/liter, κ-FLC level of 212-74,904 mg/liter, and λ-FLC level of 575-34,784 mg/liter. For two patients with normal urea and creatinine levels, extracorporeal hemocorrection was considered necessary because of high FLC levels that surpassed significantly the maximum allowable values and had to be reduced in order to prevent secondary complications.

Analysis of serum FLC values before treatment showed a significant increase of κ- or λ-FLC levels, sur-

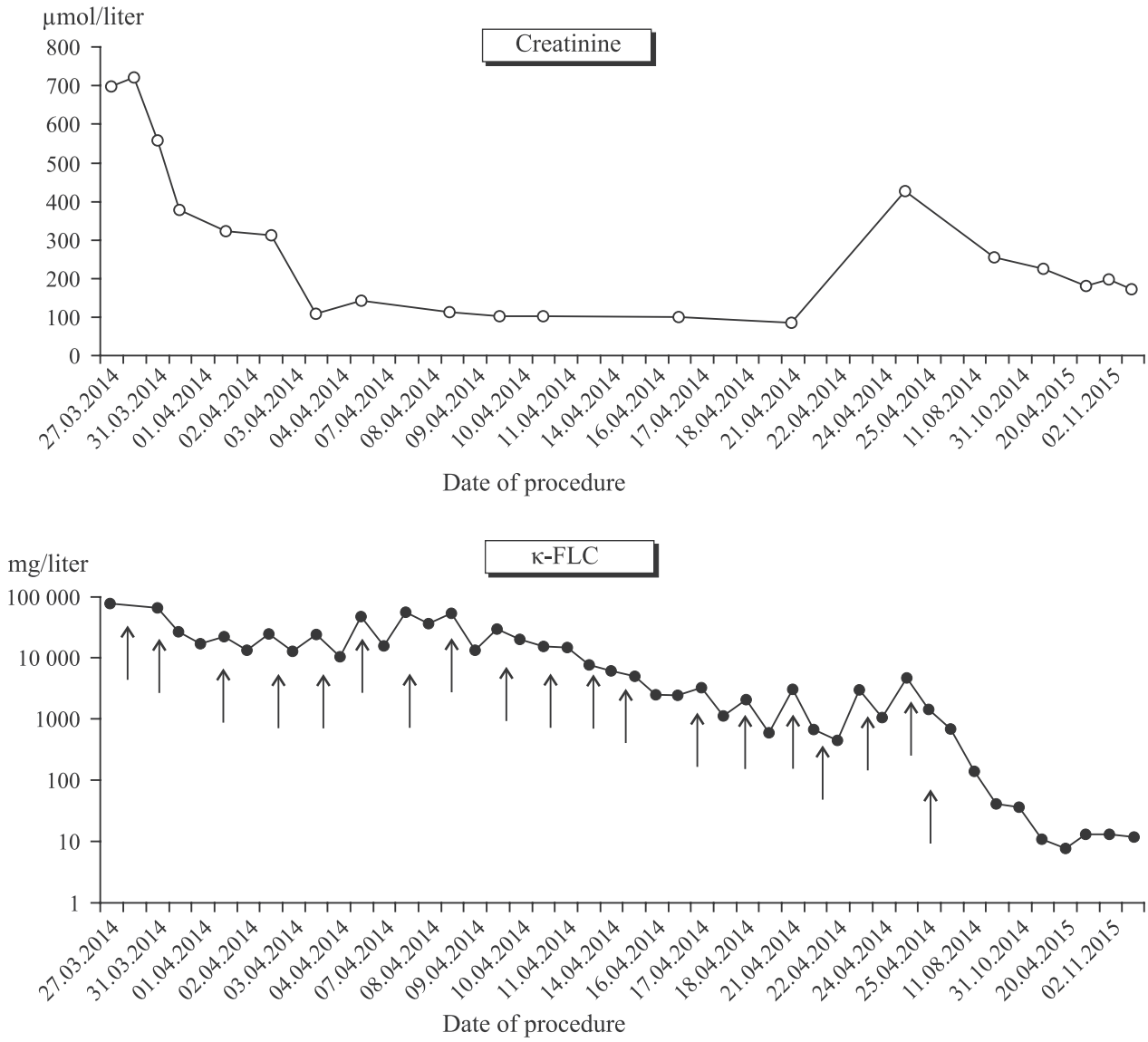

Fig. 1. Serum concentrations of creatinine and κ-FLC in patient Sh. in the course of exrtracorporeal hemocorrection. Arrows show the dates of **hemoperfusion**.

passing significantly the normal values: 3-21.5 mg/liter κ-FLC and 5-27 mg/liter λ-FLC, with κ/λ-FLC proportion of 0.25-1.65 [1,2]. The FLC medians in the group were 351 mg/liter for κ-FLC and 71.5 mg/liter for λ-FLC.

From 250 to 196,000 mg FLC could be removed during one extracorporeal detoxification procedure. The maximum volume of FLC eliminated over the entire course of extracorporeal detoxification (18 sessions) reached 1090 g. Depending on their clinical and laboratory parameters, the patients received 5 to 18 sessions with the use of EMic2 filters. Clinical tolerance of extracorporeal detoxification was satisfactory and hemodynamically stable in all patients, no traumas of blood elements or losses of serum albumin were recorded during extracorporeal treatment. Adequate antitumor therapy without drug dose reduction could be carried out in 11 patients. Renal function improved in the course of therapy in 9 patients with renal insufficiency. Ten patients exhibited a clinically significant decrease in the concentration of FLC during combined therapy. Antitumor therapy combined with extracorporeal detoxification was ineffective in one patient with initially refractory MM complicated by amyloidosis.

A clinical case demonstrates the efficiency of hemocorrection paralleled by combined therapy. Patient Sh., female, 46 years, was hospitalized with the diagnosis of MM with the κ-type IgM secretion, Bence Jones proteinuria, disseminated osteodestructive process, and soft tissue component in rib I on the right, stage IIIB. Biochemical analysis of the blood showed ure-

mia (urea 16.7 mmol/liter, creatinine 642 μmol/liter), creatinine clearance of 6 ml/min, which corresponded to dialysis-dependent stage of acute renal injury. Immunochemical studies of the serum at initial examination of the patient showed Mκ monoclonal protein and type κ Bence Jones protein (summary content 11.6 g/liter) and a significant increase of β2 microglobulin (31.9 mg/liter). Manifest hypersecretion of κ-FLC was also recorded: 74,900 mg/liter in the presence of normal λ-FLC level (8.24 mg/liter; κ/λ-FLC proportion 9090). Studies of daily urine detected type κ Bence Jones protein (3.8 g/day).

Specific antitumor therapy was carried out after extracorporeal hemocorrection aimed at reduction of FLC level and uremia. Subsequent polychemotherapy included bortesomib, cyclophosphamide, and glucocorticoids and was paralleled by renal substitute therapy. Five induction courses and 49 extracorporeal detoxification sessions, 18 of them with the use of EMic2 selective filters, were carried out. The concentration of κ-FLC decreased after hemodiafiltration to 677 mg/liter in the presence of normal λ-FLC level (10.8 mg/liter), with the κ/λ-FLC proportion equal to 62.8. The filtration capacity of the kidneys improved: serum creatinine decreased to 256 μmol/liter, creatinine clearance was 23.8 ml/min. Induction antitumor therapy led to partial remission. Immunochemical studies of serum and urine proteins showed trace secretion of paraprotein M (0.21 g/liter) in the serum, secretion of κ-FLC was 40.9 mg/liter (in the presence of low secretion of λ-FLC — 1.5 mg/liter), with κ/λ proportion 27.3. Proteinuria reduced to 0.4 g/day, no Bence Jones protein was detected.

Young age of the patient and sensitivity to antitumor therapy allowed high-dose chemotherapy (HDCT) with transplantation of autologous hemopoietic stem cells (HSC). Stimulation of hemopoiesis by G-CSF led to production of a sufficient number of $CD34^+$ mononuclears for two courses of HDCT with subsequent transplantation of autologous HSC. Alkeran in a dose of 120 mg/m^2 was used for HDCT. The course of the posttransplantation period was in fact uneventful, and the patient was discharged from hospital in a satisfactory state with stage 2-3 chronic renal insufficiency curable by conservative methods (Fig. 1). Examination of the patient after HDCT and transplantation of autologous HSC showed complete remission with normalization of all immunochemical values. Complete remission persisted for 1.5 years by the moment of the report submission.

The study has demonstrated the efficiency of extracorporeal detoxification in patients with monoclonal gammopathies and high production of FLC: the procedure directly removes FLC excess from circulating blood irrespective of the presence and severity of renal failure. Use of EMic2 filter allows selective elimination of FLC without albumin loss. Rapid reduction of FLC level or its removal in the course of selective extracorporeal elimination can prevent the development of irreversible renal failure and make possible to carry out adequate antitumor therapy.

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