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THE IMPORTANCE OF FREE LIGHT CHAINS OF IMMUNOGLOBULINS DETERMINATION IN SERUM

ZNAČAJ ODREĐIVANJA SLOBODNIH LAKIH LANACA IMUNOGLOBULINA U SERUMU

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Summary: For many years, Bence Jones proteinuria has been an important diagnostic marker for multiple myeloma. Relatively new serum tests for free kappa and free lambda light chains of immunoglobulins reflect the production of free light chains more accurately than urine tests. In this study, we examined the value of serum free light chains measurement in the diagnosis of some neoplastic diseases and the discrepance between the findings of serum protein electrophoresis and serum free light chains. Thirty one patients (f=19, m=12) were included in the study, most of them with blood malignant diseases. The results show that in six patients with normal gamma and beta electrophoresis fractions there are abnormal levels of free light chains and/or an abnormal κ/λ ratio. In 20 patients we found an abnormal κ/λ ratio, and in 21 patients we found an abnormal κ or λ level, or both. The obtained results show the important role of serum free light chains determination in identifying patients with monoclonal gammopathies.

Keywords: serum free light chains, serum protein electrophoresis

Kratak sadržaj: Bence Jones proteinurija se već dugo upotrebljava kao važan marker u dijagnostici multiplog mijeloma. Relativno novi serumski testovi za određivanje slobodnih κ i λ lakih lanaca imunoglobulina bolje reflektuju produkciju lakih lanaca od urinskih testova. U ovoj studiji ispitivali smo značaj određivanja slobodnih lakih lanaca u serumu u sklopu dijagnostike pojedinih malignih bolesti, kao i diskrepancu između nalaza serumskih lakih lanaca i elektroforeze serumskih proteina. U studiju je uključen 31 pacijent (ž=19, m=12). Većina ovih pacijenata obolela je od neke od malignih bolesti krvi. Rezultati pokazuju da kod 6 pacijenata sa urednim vrednostima gama i beta frakcije elektroforeze serumskih proteina postoje abnormalne vrednosti serumskih lakih lanaca i/ili abnormalni κ/λ odnos. Ovaj odnos bio je poremećen kod 20 pacijenata, dok je kod 21 pacijenta bio povećan nivo ili κ ili λ slobodnog lakog lanca ili oba istovremeno. Dobijeni rezultati ukazuju na značajnu ulogu određivanja serumskih koncentracija slobodnih lakih lanaca imunoglobulina u dijagnostici monoklonskih gamapatija.

Ključne reči: serumski slobodni laki lanci, elektroforeza serumskih proteina

Introduction

For more than 150 years, the presence of Bence Jones protein [immunoglobulin free light chains (FLCs)] in the urine has been an important diagnostic marker for multiple myeloma. Indeed, it was the first cancer test, and 100 years before any others (1). Development of serum tests for free κ and free λ has opened the door to new applications and increased their clinical importance (2).

Antibody molecules are composed of two identical heavy chains and two identical light chains. There are two types of light chains, κ and λ. Any given antibody molecule has either type of light chain, but never both. The variable domains of each light

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List of abbreviations:
FLCs – immunoglobulin free light chains; SPE – serum protein electrophoresis; MM – multiple myeloma; BJ – Bence Jones; NHL – Non-Hodgkin lymphoma; CLL – chronic lymphocytic leukaemia; κ and λ – kappa and lambda immunoglobulin free light chains; SLE – systemic lupus erythematosus

chain, heavy chain pair combine to form an antigen-binding site. FLCs are incorporated into immunoglobulin molecules during B lymphocyte development and expressed initially on the surface of immature B-cells. Production of FLCs occurs throughout the rest of B-cells development and in plasma cells, where secretion is highest (3).

Production of FLCs in normal individuals is approximately 500 mg/day from bone marrow and lymph node cells (4). Immunoglobulin light chains and heavy chains are combined together during the synthesis of immunoglobulins; however, more light chains than heavy chains are produced. Thus, light chains that are not bound to intact immunoglobulins can be detected as circulating FLCs under physiological conditions (5). There are twice as many κ producing plasma cells as λ. Kappa FLCs are normally monomeric, while λ FLCs tend to be dimeric.

Under ordinary circumstances, little protein escapes to the urine, and serum FLCs concentrations have to increase manyfold before the absorption mechanisms are overwhelmed and before urine contains significant amounts of FLCs (6). In normal individuals, 1–10 mg of FLCs is excreted per day into the urine. Production rates of κ are twice those of λ, but κ FLCs monomers are cleared three times faster than dimeric λ molecules (7). This faster removal ensures that actual serum concentrations of κ are approximately 50% lower than of λ. In normal individuals, serum FLCs are cleared rapidly through the renal glomeruli with a serum half-life of 2–6 h and are then metabolized in the proximal tubules of the nephrons (8). In contrast, IgG has a half-life of 21 days. Removal may be prolonged to 2–3 days in multiple myeloma patients in cases of complete renal failure (9), when serum levels of FLCs rise rapidly, but urine excretion falls (10). Consequently, the levels of serum and urine FLCs diverge during the later stage of disease.

A new automated immunoassay now allows for sensitive and specific FLC assessment using antibodies directed against the »hidden« epitopes of FLC molecules, located at the interface between the light and heavy chains of intact immunoglobulins (11). To date, this assay has essentially been used to assess the excess of one light chain over another, using κ/λ ratio as a surrogate for clonal expansion. Thus, assessment of quantitative FLC levels already represents a diagnostic test in the routine monitoring of monoclonal gammopathy: non-secretory myeloma (12), light-chain myeloma (6), primary amyloidosis (13) and monoclonal gammopathy of undetermined significance (MGUS) (14). Other indications include: a. rapid assessment of treatment responses, b. monitoring MM patients in renal failure undergoing haemodialysis (15), c. monitoring the patients that cannot be assessed by electrophoresis tests. Serum protein electrophoresis (SPE) is the standard screening method for multiple myeloma, but sensitivity of SPE for FLC detection is between 500 mg/L and 2 g/L (2). As a general rule, intact immunoglobulin monoclonal proteins can be identified using SPE, while monoclonal light chain diseases should be identified using serum FLC assay.

Serum FLCs in combination with SPE and serum immunofixation electrophoresis identified >99% of patients with monoclonal gammopathies. Patients' results are separated into different categories depending upon several factors: whether the clone is κ or λ, the presence of renal failure or polyclonal hypergammaglobulinaemia and the degree of bone marrow impairment from the growing tumour or from drug therapy.

The rules for the interpretation of serum free light chain results are as follows (3):

A. Normal values – serum levels of κ, λ and κ/λ ratio are all within the normal ranges. In combination with normal SPE, it is most unlikely that the patient has a monoclonal gammopathy.

B. Abnormal κ/λ ratio – supports the diagnosis of monoclonal gammopathy and requires a tissue biopsy. Borderline elevated κ/λ ratios occur with renal impairment and may require appropriate renal function tests.

C. Low concentrations of κ, λ or both – indicate bone marrow function impairment.

D. Elevated concentrations of both κ and λ with abnormal κ/λ ratio – may be due to renal impairment (common) or overproduction of polyclonal FLCs caused by inflammatory conditions (common) or biclonal gammopathies of different FLC types (rare).

E. Elevated concentrations of both κ and λ with an abnormal κ/λ ratio – suggest a combination of monoclonal gammopathy and renal impairment.

The aim of the present study was to evaluate the value of serum free light chains determination in the diagnosis of monoclonal gammopathies.

Material and Methods

In this cross-sectional study, thirty one patients (19 females and 12 males) were studied. The structure of patients is shown in *Table I*.

Serum FLCs and SPE were recorded in all patients. Serum FLCs levels were measured using a latex-enhanced immunoassay (Freelite, UK) on an Olympus AU400 turbidimetry analyzer. The immunoassay consisted of two separate measurements, one to detect free κ (normal range: 3.3–19.4 mg/L) and the other to detect free λ (normal range: 5.7–26.3 mg/L). A ratio of κ/λ <0.26 or >1.65 is abnormal. Serum protein electrophoresis were determined using an

Table I Established diagnosis of patients.

Disease	Number
Multiple myeloma	10
Chronic lymphocytic leukaemia	4
Non-Hodgkin lymphoma	4
Plasmocytic leukaemia	1
M. Hodgkin	2
Mycosis fungoides	1
Myelofibrosis	1
Splenomegalia	1
Asplenia	1
Autoimmune haemolytic anemia	1
Chronic renal failure	2
IgA deficit	2
IgG deficit	1
Vasculitis	1
Oligoarthritis	1

Olympus HITE system (zone electrophoresis). This system performs separation of proteins on the cellulose acetate underlying membrane. Olympus HITE system automatically calculates distribution of protein groups and results are expressed as relative units (%).

Results

Figure 1 represents the structure of patients, concerning the serum values of κ and λ free light chains.

In the group with an abnormal κ/λ ratio, two patients had regular values of both light chains, two had low values of one light chain, and one of them had increased values of both light chains. The rest of the patients (n=15) had increased values of one light chain. In the group of patients with high values of one or both FLCs, concentrations ranged from 31.93 mg/L to 3275.6 mg/L. The values of κ ratio ranged from 0.002 to 1136.

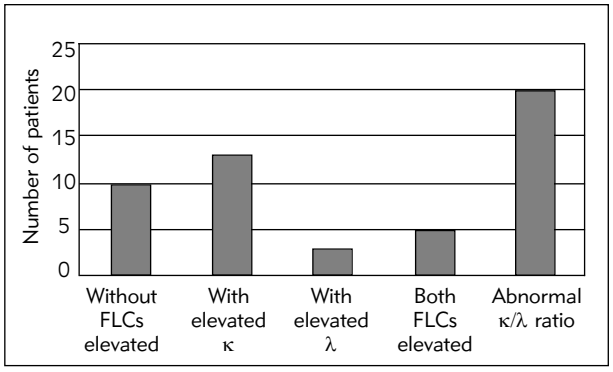

Figure 1 FLCs – free light chains of immunoglobulins.

In the group of patients with high concentrations of one of FLCs, we can see that 6 of them had neither increasing gamma nor beta fractions of SPE. The values of κ or λ chains in these patients were from 39.57 to 2703.8 mg/L. In one patient with high values of both FLCs there were no changes in these two fractions of SPE. Among the rest of 14 patients with high values of κ and/or λ, the increasing of gamma or beta fractions of SPE was noticed.

Discussion

Plasma cell dyscrasias are characterized by expansion of a single clone of immunoglobulin-producing plasma cells and a resultant increase in serum levels of a single monoclonal immunoglobulin or its fragments. Multiple myeloma is by far the most frequent of the malignant plasma cell dyscrasias, accounting for 1% of all cancers and 10% of all hematologic malignancies in whites (16). One of the characteristics of multiple myeloma is the unregulated production of a monoclonal antibody referred to as the *M protein* because it is detected as an M spike on SPE. Usually, this M component is in the gamma region, rarely in the β_2 region of the SPE. In most cases, the M protein is either IgG (60%) or IgA (20–25%) (17). In the remaining 15% to 20% of cases, the plasma cells produce only Bence Jones proteins, abnormal proteins that consist of light chains of the immunoglobulin molecule. Persons with this form of disease (light chain disease) have Bence Jones proteins in their serum, but lack the M component. In our study, we met such findings in some patients, who had high serum concentrations of κ or λ, and lacked the M component. Concentrations of κ or λ in these patients ranged from 39.57 to even 2703.8 mg/L. However, up to 80% of myeloma cells produce both complete immunoglobulins and excess light chains; therefore, both M proteins and Bence Jones proteins are present (16). In this study, as was expected, we found such patients. M component can be found in other neoplastic and non-neoplastic diseases: chronic lymphocytic leukemia (CLL), B or T-cells lymphoma, chronic granulocyte leukemia, colon cancer, autoimmune diseases, cirrhosis, parasitic diseases, sarcoidosis.

When there is increased polyclonal immunoglobulin production and/or renal impairment, both κ and λ FLC concentrations can increase 10- to 20-fold. However, the relative concentration of κ to λ, i.e., the κ/λ ratio, remains unchanged. In our study we found one patient with advanced chronic renal failure. Kappa and λ concentrations were increased many-fold (κ: 238.9 mg/L and λ: 186.8 mg/L), but κ/λ ratio was normal: 1.27. Reduced clearance of

FLCs that is a result of impaired renal glomerular filtration is a frequent finding, and can be seen even in apparently healthy, elderly individuals who may have normal serum creatinine concentrations but elevated polyclonal serum FLCs from slight renal damage. This is associated with an increase in the median κ/λ ratio in older people (3). The change in κ/λ ratio with increasing renal impairment is of clinical relevance when interpreting borderline results. Patients might be misclassified as having minor κ monoclonal gammopathies because of slightly increased κ/λ ratios, when, in fact, they have renal impairment. Cystatin C concentrations have very high correlation with FLC levels (11). It is likely that many patients with chronic inflammatory diseases and associated renal impairment will be found to have very high concentrations of polyclonal serum FLCs, but with normal κ/λ ratio. The clinical consequences of elevated serum FLCs in renal impairment are unclear. Data have suggested that the elevated FLCs lead to reductions in immune functions and should therefore be classified as uraemic toxins (18).

Tumors produce a monoclonal excess of only one of the light chains, often with bone marrow suppression of the other light chain, so that κ/λ ratios become highly abnormal. Accurate measurement of κ/λ ratios underpins the utility of the serum FLC immunoassays and provides a numerical indicator of clonality (11, 26). The κ/λ ratio is the most important factor when distinguishing monoclonal from polyclonal increases in FLCs. In our patients κ/λ ratios were abnormal in 20 patients, even in patients with normal concentrations of κ and λ. This ratio ranged from 0.002 to 1136, depending on the type of FLC disturbance, and in patients with very high levels of one of FLCs the other was usually low. We found one patient with normal κ level and low λ level with abnormal κ/λ ratio. That finding can suggest monoclonal gammopathy with bone marrow suppression (3).

Measurement of serum FLCs can be used in other malignancies with monoclonal proteins: to detect and monitor most patients with solitary plasmacytomas of the bone (19); it may be helpful in managing some of solitary plasmocytoma patients; it may be helpful in Waldenstrom's macroglobulinaemia: when IgM measurements are inaccurate, or to identify patients at risk of renal failure and as additional criteria for treatment response or disease relapse (3); in Non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukaemia (CLL) patients serum FLCs may enable early diagnosis, and serve as a biomarker of residual disease – particularly important for NHL, because most patients with NHL have no circulating malignant cells, unlike CLL, where a small number of circulating leukemic cells often exists that can be identified using sensitive flow cytometry techniques (20). In our study, some of the patients with lymphoproliferative diseases had normal and some had abnormal concentrations of FLCs (the highest concentration of κ or λ was 194.3 mg/L).

Increased serum level of FLCs (polyclonal) is associated with a generalized increase (non-malignant) in B-cell activation. The interest in B cell activation markers has undergone a renaissance over the past few years, given the pivotal role of B cells in the pathogenesis of autoimmune diseases (21). Many rheumatic diseases feature polyclonal B-cell activation, high concentration of autoimmune antibodies and polyclonal elevations of serum immunoglobulins. Excess polyclonal FLCs have been detected in the urine of these patients, and their measurement may be useful for assessing disease activity. In fact, serum analysis of FLCs in such patients would be more reliable. This may be particularly applicable to patients with systemic lupus erythematosus (SLE) (22). Hoffman et al. (23) found high FLC concentrations in rheumatoid arthritis, Sjögren's syndrome – patients with increased FLC levels had considerably higher disease activity in rheumatoid arthritis and higher frequency of extraglandular involvement in primary Sjögren's syndrome (5), vasculitis and systemic sclerosis. There are studies of serum FLCs in other acute or chronic inflammatory diseases (24), and a recent study of a few patients with acute pneumonia, where polyclonal serum FLCs were elevated. In our study, one patient had a combination of oligoarthritis and IgA deficit. Concentrations of κ and λ were slightly above the reference interval, but with normal κ/λ ratio.

Diabetic patients have significant FLC abnormalities in serum and urine that may be involved in the pathogenesis of diabetic nephropathy. Increased urine FLCs may be a marker of early diabetic nephropathy. FLCs are also raised in the cerebrospinal fluid (CSF) of patients with multiple sclerosis. Fisher et al. concluded that CSF κ FLC measurements may be a useful diagnostic procedure for detecting, and potentially monitoring, intrathecal immunoglobulin synthesis (25).

In conclusion, determination of FLCs plays an important role in the diagnostics of monoclonal gammopathies tests, but, in accordance with the literature data and the results of this investigation, this test may aid in the diagnosis of some other diseases: lymphoproliferative diseases, SLE, multiple sclerosis, etc.

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