*Iwona Grygoruk-Wiśniowska, Sławomira Kyrcz-Krzemień

An analysis of discrepancies between test results of the selected protein parameters and clonal bone marrow plasma cells in an assessment of treatment response in a group of patients with multiple myeloma (MM) prior to an autologous hematopoietic stem cell transplantation (AHSCT)

Analiza niespójności wyników badań wybranych parametrów białkowych surowicy krwi i klonalnych plazmocytów szpiku w ocenie odpowiedzi na leczenie u chorych ze szpiczakiem plazmocytowym (MM) poddanych autologicznej transplantacji krwiotwórczych komórek macierzystych (AHSCT)

Department of Haematology and Bone Marrow Transplantation, Medical University of Silesia, Katowice
Head of Department: prof. Sławomira Kyrcz-Krzemień, MD, PhD

Key words

multiple myeloma, electrophoresis, immunofixation, Freelite, discrepancy, autologous hematopoietic stem cell transplantation, prognosis

Słowa kluczowe

szpiczak plazmocytowy, elektroforeza, immunofiksacja, Freelite, dyskrepancja, autologiczna transplantacja macierzystych komórek hematopoetycznych, rokowanie

S u m m a r y

Introduction. Multiple myeloma (MM) is an incurable, B-cell malignancy. Modern diagnostics and disease monitoring include the assessment of serum and urine protein as well as bone marrow examination. The discrepancies of the results provided by these studies may cause the difficulties in the interpretation of disease status.

Aim. The analysis the incompatibility of test results a selected protein parameters and clonal plasma cells in bone marrow in response to treatment in MM patients prior to the AHSCT.

Material and methods. Seventy two MM patients (37 male and 35 female) at a median age of 58 years (range 38-81 years) were included in this study. The following diagnostic tests have been used: electrophoresis (SPE), immunofixation (IFE), Freelite for serum free light chains kappa and lambda assessments and clonal plasma cell analysis in bone marrow by flow cytometry (FC).

Results. 32 patients (44%) were found to have discrepancies in the diagnostic tests evaluating disease status before transplant; in the remaining 40 patients (56%) the results were consistent. The 4 types of discrepancies were detected.

Conclusions. At least several diagnostic tests are required in order to reliable assess the MM status before AHSCT and clonal plasma cell assessment by FC may remain an additional diagnostic tool.

S t r e s z c z e n i e

Wstęp. Szpiczak plazmocytowy (ang. *multiple myeloma* – MM) jest nieuleczalną chorobą rozrostową układu krwiotwórczego wywodzącą się z limfoidalnych komórek B. Współczesna diagnostyka i monitorowanie MM obejmuje testy białkowe surowicy i moczu oraz badanie cytologiczne aspiratu szpiku kostnego. Niespójności uzyskiwanych wyników mogą być przyczyną trudności interpretacyjnych.

Cel pracy. Analiza niespójności wyników badań wybranych parametrów białkowych surowicy krwi i klonalnych plazmocytów szpiku u chorych ze szpiczakiem plazmocytowym przed zabiegiem AHSCT.

Materiał i metody. Do badania włączono 72 chorych z MM ocenianych przed rozpoczęciem procedury AHSCT. Analizą objęto 37 mężczyzn i 35 kobiet w medianie wieku 58 lat (zakres 38-81 lat). Wykorzystano następujące testy diagnostyczne: elektroforezę białek surowicy (SPE), immunofiksację (IFE), test Freelite do oceny stężenia wolnych lekkich łańcuchów kappa, lambda i ich wzajemnego stosunku oraz badanie klonalności plazmocytów szpiku przy użyciu cytometrii przepływowej.

Wyniki. Spośród 72 pacjentów poddanych analizie u 32 (44%) stwierdzono występowanie niespójności analitycznych w testach diagnostycznych stosowanych w ocenie

Address/adres:

*Iwona Grygoruk-Wiśniowska
Department of Haematology
and Bone Marrow Transplantation
Medical University of Silesia
ul. Dąbrowskiego 25, 40-032 Katowice
tel./fax +48 (32) 259-12-81
klinhem@sum.edu.pl

stanu remisji przed AHSCT. U 40 (56%) pacjentów wyniki testów diagnostycznych były spójne. Zaobserwowano cztery typy niespójności.

Wnioski. Właściwa ocena stanu remisji szpiczaka plazmocytowego przed zabiegiem AHSCT wymaga wykorzystania co najmniej kilku testów diagnostycznych ze względu na obserwowane niespójności, a badanie klonalności plazmocytów przy zastosowaniu cytometrii przepływowej stanowi jedynie metodę uzupełniającą.

INTRODUCTION

Multiple myeloma (MM) is an incurable, B-cell malignancy. MM represents about 10% of all hematologic malignancies and is characterized by the proliferation of single clone plasma cells (1).

The first description of the disease comes from the 80's of the Nineteenth Century and was written by Samuela Sollye, John Dalrymple and Henry Bence-Jones. In 1889 Otto Kahler and Osip Rusticki used the term myeloma multiplex for the first time (2).

The annual incidence rate is 4-7 cases per 100 000 inhabitants. It affects slightly more men and people of certain races, such as African or Afro-Caribbean. Furthermore, the average age of the diagnosis is 65 (3).

The etiology of multiple myeloma is not clear. The causes of the disease are complex, probably following various stages and then leading to gene mutations. The development of MM is closely connected to clonal plasma cells which secrete many autocrine substances and stimulating factors such as: MIPIα, MIPIβ, TNF, M-CSF, HGF, VEGF, MMP9, MMP2, IL-1, IL-3, IL-6, IL-11 (4, 5).

Major clinical manifestations are: renal failure, hypercalcemia, anemia, osteolitic bone lesion or pathological fractures. Non-specific symptoms are: weakness, weight loss, fever, susceptibility to infection, neuropathy (6).

Each case of MM is preceded by an asymptomatic malignant stage, termed MGUS – monoclonal gammopathy of undetermined significance. MGUS is characterized by the presence of the M protein in serum (< 30 g/L) and the appearance of clonal plasma cells in the bone marrow which is greater than or equal to 10% (7). The risk of progression to myeloma is 1% per year (8). The next stage in the development of MM is smoldering or asymptomatic multiple myeloma (SMM). In order to identify SMM, monoclonal protein concentration must be over 30 g/L and/or clonal plasma cells in the bone marrow must exceed 10% in the absence of end-organ damage (CRAB symptoms). The estimated risk of progression of SMM to multiple myeloma is on the level of 10% per year for the first 5 years since recognition (9).

A number of tests are used to help confirm multiple myeloma. The diagnosis require the presence of the clonal plasma cell in the bone marrow (> 10%), the presence of monoclonal proteins in the serum and/or urine and so-called CRAB symptoms.

International Myeloma Working Group has established criteria for the diagnosis and monitoring response to treatment in patients with monoclonal gammopathy. This organization recommends the following diagnostic tests performed in the serum and urine: electrophoresis (SPE), immunofixation (IFE), a serum kappa and lambda free light chains assessment along with a flow cytometry analysis (FC) of clonal bone marrow plasma cells (10-12).

AIM

An analysis of discrepancies in the test results of the selected protein parameters and clonal bone marrow plasma cells in MM patients prior to the AHSCT.

MATERIAL AND METHODS

Seventy two MM patients (37 male and 35 female) with a median age of 58 years (range 38-81 years) were included in this study. All of them were treated with chemotherapy in the Department of Haematology and Bone Marrow Transplantation Silesian University in Katowice, between 2011 and 2013. The following diagnostic tests were used: electrophoresis (SPE), immunofixation (IFE), a serum kappa and lambda free light chains assessment (Freelite) and a flow cytometry analysis (FC) of clonal plasma cells in the bone marrow.

RESULTS

Out of 72 patients, 32 (44%) were found to have discrepancies in the diagnostic tests evaluating disease status before the transplant; in the remaining 40 patients (56%) the results were consistent.

4 types of discrepancies were detected: 1 – Freelite and FC tests were negative, but SPE/IFE was positive (n = 10) (tab. 1, item 1-10), 2 – Freelite test was negative whereas FC and SPE/IFE were positive (n = 10) (tab. 1, item 11-20), 3 – FC was negative with positive Freelite test and SPE/IFE (n = 8) (tab. 1, item 21-28) and 4 – FC and SPE/IFE were negative with positive Freelite test (n = 4) (tab. 1, item 29-32).

The analysis of patients with MM identified two subgroups: Group A – without the presence of discrepancies in the study and Group B – with the presence of discrepancies in the study.

Considering parameters such as: age, sex, multiple myeloma subtype, response to treatment, type of treatment, the number of therapy lines, blood morphology parameters, recovery times in each line after AHSCT and duration of the disease – no significant differences were proved between the parameters of the patients from group A (without the presence of discrepancies) and those from group B (with the presence of discrepancies). The comparison of the MM

Table 1. The discrepancies in the results of the diagnostic tests.

Patient	Clonal plasma cells	FLCr (FLCsκ/FLCsλ)	IFE	SPE
1	0	0	1	1
2	0	0	1	0
3	0	0	1	1
4	0	0	1	1
5	0	0	1	1
6	0	0	1	0
7	0	0	1	1
8	0	0	1	0
9	0	0	1	0
10	0	0	1	1
11	1	0	1	0
12	1	0	1	1
13	1	0	1	0
14	1	0	1	1
15	1	0	1	1
16	1	0	1	1
17	1	0	1	0
18	1	0	1	1
19	1	0	1	0
20	1	0	1	0
21	0	1	1	1
22	0	1	1	1
23	0	1	1	0
24	0	1	1	0
25	0	1	1	1
26	0	1	1	1
27	0	1	1	0
28	0	1	1	0
29	0	1	0	0
30	0	1	0	0
31	0	1	0	0
32	0	1	0	0

FLCr – FLC ratio κ/λ; IFE – serum protein immunofixation; SPE – serum protein electrophoresis
0 – negative result; 1 – positive result

patients whose tests results did not have discrepancies in protein tests (group A) with the patients whose tests had discrepancies (group B) was illustrated in table 2.

DISCUSSION

Multiple myeloma is a disease that is characterized by the proliferation and accumulation of clonal plasma cells producing monoclonal protein (1).

For over one and a half centuries, the presence of Bence Jones protein has been the most important diagnostic marker of monoclonal gammopathy (13).

In 2001, a special test called the serum free light chain (FLC) assay or Freelite test was introduced into clinical practice. Since then, it has been the standard test performed in patients with MM, used in diagnosis, prognosis, monitoring of response to treatment and, finally, for a quick evaluation of recurrence/progression (14).

The next quantum leap in the history of research on multiple myeloma was the discovery of the free light chains escape phenomenon which involves an increase of FLCs in patients with relapsing multiple myeloma, while still maintaining normal serum immunoglobulin levels. The free light chains escape is associated with the proliferation of the plasma cells clone producing only light chains and is not related to the clone producing complete immuglobin molecule. This leads to a significant increase in the concentration of FLCs in the serum, while that of the complete immunoglobulin level is not altered. Early detection of the free light chains escape is of clinical relevance for several reasons:

- it is related to a significant increase in tumor weight hence is used to measure the severity of the disease,
- it helps to identify patients with a high risk of progression and poor prognosis,
- it helps to avoid complications associated with the development of renal failure (15).

Discrepancies in the results of protein tests used in the evaluation and follow-up of patients with multiple myeloma, are the subject of numerous reports (16-20).

The presence of protein discrepancies in patients with monoclonal gammopathy was described by Singhal et al., in the report from 2009. While analyzing a group of 122 patients with MM IgG and IgA, the authors observed that in the case of 34% of patients, the proper ratio of FLCs is accompanied by the presence of monoclonal protein in the IFE (21). These findings appear to support the same thesis as one included in my studies where the test results had similar discrepancies in 18 patients (25%).

Other studies about discrepancies in laboratory test results were also published in the following years. The results of 280 patients with multiple myeloma were compared using the following methods: IFE, Freelite and flow cytometry. 7 out of all the patients (10%) had discrepancies in the results of laboratory tests, that is, the presence of monoclonal protein in the IFE was accompanied by the absence of clonal plasma cells in the flow cytometry (22). Similar discrepancies were found in my tests, where they occurred in 18 patients (25%).

Further, in 2013, there appeared the next publication which analyzed 109 patients with newly diagnosed multiple myeloma, comparing the results obtained by electrophoresis and the Freelite test. Discrepancies were observed in 11 patients (10%), in 7 patients (6%) the proper FLCs concentration was accompanied by the presence of a monoclonal protein in electrophoresis and immunofixation, in 4 patients (4%) there was found the abnormal value of FLCs in the absence of M protein in the above diagnostic assays (23). In this study, similarly to those

Table 2. The comparison of the MM patients whose tests results did not have discrepancies in protein tests (group A) with the patients whose tests had discrepancies (group B).

Parameter	Group A (n = 40)	Group B (n = 32)	p
Age (median, range); years	58.2 (44-74)	58.7 (38-80.5)	0.27
Sex (W/M) (n (%))	19 (47%)/21(53%)	16 (50%)/16 (50%)	0.99
Subtype MM (n (%)): IgG kappa IgG lambda IgA kappa IgA lambda	 19 (48%) 9 (22%) 8 (20%) 4 (10%)	 16 (50%) 11 (35%) 3 (9%) 2 (6%)	0.13
Disease state (n (%)): sCR CR VGPR PR	 6 (15%) 7 (18%) 10 (25%) 17 (42%)	 0 (0%) 4 (13%) 20 (63%) 8 (25%)	0.90
Number lines of the treatment (median, range)	1 (1-4)	1 (1-4)	0.41
Induction therapy CTD (n (%))	31 (78%)	27 (84%)	0.42
RBC (x $10^6/\mu$L) (median, range)	4.1 (3-5.1)	4.1 (3.1-4.7)	0.37
HCT (%) (median, range)	38 (31-45)	38 (29-42)	0.62
Hgb (g/dL) (median, range)	12.8 (10.4-16)	12.4 (9.8-14.9)	0.74
PLT (x 10^9L) (median, range)	207 (76-296)	208 (93-358)	0.16
WBC (x 10^9/L) (median, range)	4.4 (2.5-9.7)	4.5 (2-9.7)	0.91
Regeneration WBC (days) (median, range)	12 (8-20)	13 (10-17)	0.31
Regeneration ANC (days) (median, range)	13 (9-20)	13 (10-18)	0.69
Regeneration PLT (days) (median, range)	10 (8-17)	11 (8-17)	0.48
Time to AHSCT (months) (median, range)	19 (8-149)	14 (8-71)	0.89
Time from diagnosis to the last follow-up (months) (median, range)	45 (23-168)	26 (17-83)	0.79

ANC – absolute neutrophil count; CTD – cyclophosphamide , thalidomide, dexamethasone; CR – complete response; HCT – haematocrit; Hgb – hemoglobin concentration; K– woman; M – man; PLT – platelet count; PR – partial response; RBC – red blood cell count; sCR – stringent complete response; WBC – white blood cells count; VGPR – very good partial response

described above, the inconsistencies were observed in 18 (25%) and 4 patients (6%).

The analysis of a group of 72 patients based on parameters as: age, sex, multiple myeloma subtype, response to treatment, type of treatment, the number of lines of therapy, blood cell counts parameters, recovery times in each line after AHSCT and duration of the disease; did not show significant differences between the parameters studied in patients for myeloma whose test results had discrepancies (group A) and patients whose results did not have discrepancies (group B).

With regard to the test results, it is important to question the cause of the observed discrepancies.

Plasma cells and B lymphocytes in bone marrow and lymph nodes produce one out of five types of heavy chains, including one of the light chains: κ or λ. In contrast to κ chains, which are often found as monomers, λ chains have a dimeric form (24).

The concentration of the serum monoclonal protein produced and secreted by plasma cells is a result of the balance between its production by a particular tumor mass and excretion by the kidneys and a period of its half-life (25, 26).

The half-life depends on the size of the molecule and the efficiency of its elimination by the kidneys, and with normal creatinine clearance, it takes 2-4 hours for monomeric molecules of κ chains, 3-6 hours for dimeric molecules of λ chains and 2-3 days for particles having a larger polymerization. As it approaches the half-life of complete molecules, it takes 3 days for IgD, 5 days for IgM, 20-25 days for IgG and 6 days for IgA (27). The production of FLCs in a healthy person equals about 0.5-1.0 g per day and the half-life takes 2-6 hours. They are filtered in the glomerulus of kidneys and metabolized in the proximal tubules of the nephron. Both kidney damage and polymerization of light chains increase their half-life to 2-3 days. The most frequently occurring monomeric and dimeric forms of FLCs, under the influence of various factors, e.g. chemotherapy, may transform into a polymer, and in this form the biological half-life is prolonged. The urinary excretion of FLCs in normal individuals is very slight and stands at 1-10 mg/day. They are probably secreted by the mucous membrane of the distal nephron and ureter, and along with IgA they both constitute the immune system against infections (28).

The other factor responsible for the FLCs concentration in serum is normal renal functioning. Renal failure develops in many disease states, e.g. diabetes or high blood pressure, which may appear in the course of the monoclonal gammopathy and also multiple myeloma (29).

The reduction of the renal clearance in patients with monoclonal gammopathy is caused by the increased concentration of FLCs, which damages the

distal tubule cells in the mechanism of an inflammation (FLCs connect with the Tamm-Horsfall protein and form an insoluble complex that precipitates in the distal and collecting tubules) (30-32).

Some authors suggest that diabetic nephropathy occurring with normal serum creatinine level leads to a significant loss of proteins, which exceeds their production (FLCs and albumin) (33, 34).

The proximal tubules of the nephron are able to metabolize large quantities of FLCs in a healthy person with normal renal functioning. It means that even at high concentrations of FLCs in the serum, their excretion in the urine may be slight (28). This theory was confirmed in the analysis of a group of 82 patients with MM who suffered from renal failure. The evaluation was performed with the use of three methods: SPF, IFE and Freelite. It was proved that in a given group of patients, the FLC ratio was less sensitive and specific than SPE and IFE (35). Similar findings were formulated by other research groups after analyzing patients with kidney failure (36).

The next important factor which has an impact on the concentration of FLCs is the fact that complete immunoglobulin molecules are osmotically active. A low weight of a FLC molecule (κ = 25 kDa, λ = 50 kDa), causes that only 20-30% of it is located in the vascular space, the next 20-30% in the extra-vascular space, and the remaining 20-30% in the tissue edema. MM associated lipid disorders, hematocrit level change (during the course of therapy and accompanying diseases), changes in blood volume, e.g. in the course of diabetes, cause the migration of particles which have low mass. Larger immunoglobulin molecules are less susceptible to changes of the plasma volume (37).

With the exception of MM, clonal protein might be detected in a number of diseases, both tumor diseases as well as diseases with acute and chronic inflammation. Stimulation of the B lymphocytes is a cause of the production of a mono or polyclonal immunoglobulin. Among many chronic inflammatory diseases there are: rheumatoid arthritis, systemic lupus erythematosus, primary Sjogren's syndrome, chronic hepatitis C, atopic dermatitis, infection of Helicobacter pylori. The acute inflammatory conditions include: acute pneumonia or neuroinfection. The polyclonal protein can cause damage to the glomeruli, filtration disorders, as well as obtaining false concentrations of monoclonal FLCs (38, 39).

The analysis of 34 patients with MM who were transplanted in complete remission, demonstrated that in 14 of them the FLCr value was incorrect, due to the presence of a polyclonal protein (40).

The neoplastic diseases associated with the presence of a monoclonal protein are: other monoclonal gammopathies/atypical forms of multiple myeloma, Waldenstrom's macroglobulinemia, B-cell non-Hodgkin's lymphoma, chronic lymphocytic leukemia, cryoglobinemia (monoclonal or polyclonal), and many others (41).

In a small population of patients with multiple myeloma (1-2%) it is possible to prove the presence of a biclonal protein, namely the presence of both kappa and lambda FLCs. In this case, the result of FLCr can remain in the normal range (42). A similar situation can be found in non-secretory MM, where the FLCr can be at a range of the norm (43).

Monoclonal protein is measured using three methods. Each of them has a different sensitivity and specificity. Two of them are quantitative methods – electrophoresis and Freelite, one is a qualitative method – IFE. Freelite is the most sensitive diagnostic quantitative test (44).

The studies indicate that FLCs are characterized by high sensitivity (they are incorrect in 96-97% of patients) and have a predictive value. They are an early and rapid marker of the efficacy of treatment, remission, residual disease and relapse. There are described cases of different results for the quantitative determination of free light chains depending on the test used. In 2013 there was published a comparative study of the FLC results in a group of 327 patients who had had Freelite and N latex tests. The results were comparable for FLCs kappa (81%) and FLCs lambda (74%) (45). Another test was performed in a group of 541 patients. A comparative analysis of the results was carried out by the use of Frelite, N latex and immunofixation. Compliance of these three methods was on the level of 95-97% (46). The analysis based on 9 patients with multiple myeloma aged 61-80 and a group of 170 healthy volunteers aged 17-66, showed a 20% discrepancy in values for the FLCs kappa and FLC ratio for people who were over 60 years old (47).

A great advantage of the Freelite test is the short period needed for the obtaining of results as well as the stability of the collected FLCs in a properly stored serum sample (48).

Immunofixation, as a qualitative method, can be interpreted subjectively and therefore is the only method that cannot be used to monitor the treatment. The designation of proteins by means of this method depends on the skills and experience of the staff performing the test. It happens that the polyclonal protein arranged in the form of a broad diffuse band can cause the masking of a narrow and often discrete monoclonal protein band. In this method, the calibration is performed with the use of polyclonal reagents, but they may differ with the epitopes of monoclonal forms (different series of reagent). This may lead to discrete changes in the calibration range. A high sensitivity of serum immunofixation is estimated according to various observations multiple at more than 90% (49, 50).

Electrophoresis allows one to detect even weak zones of monoclonal protein, especially if they are in a typically gamma globulin location. We then find present in the protein fraction a characteristically monoclonal protein band which is typically located in the region of the gamma or beta, less often in the region of the alpha-2. The height of a peak (marked densi-

tometrically) is proportional to the concentration of a monoclonal protein. The electrophoresis indicates the presence of a monoclonal protein fraction in approximately 80-90% of patients with myeloma. Electrophoresis results can be difficult to interpret. When the weak zone of monoclonal protein is located abnormally (near beta fraction, within the beta or even alpha-2 globulin) there may appear densitometry mistakes. Sometimes monoclonal protein migrates with other serum proteins (in this case monoclonal protein cannot be measured) (51, 52). The protein concentration of monoclonal protein can be underestimated up to even 30%. The main reason of this is the dye gel saturation (10). The sensitivity of the SPE is also limited, when the protein M concentration is below 20 g/L – the lower the concentration, the bigger the variability. The analysis of 2845 patients with MM with the use of two different methods, that is either the agarose gel electrophoresis or the immunonephelometric method, showed differences in the quantitative determination of immunoglobulins (53).

The other factor affecting the presence and the concentration of serum monoclonal protein is a tumor mass which correlates with the disease remission state. The presence of clonal plasma cells is one of the elements of the diagnosis and monitoring of multiple myeloma, and their number correlates with the severity of the disease and is considered a predictor of survival (54). Previous studies have shown that the concentration of FLCs and FLCr correlates with the percentage of clonal plasma cells in the bone marrow (55).

In my analyzed group of patients, the percentage of clonal plasma cells did not have an impact on the disease-free survival. Out of 32 patients with the presence of diagnostic discrepancies, 69% did not prove the presence of clonal plasma cells despite the positive protein tests.

The assessment of clonal plasma cells is performed after iliac spine or sternum aspiration. Generally, plasma cell infiltration is focal or often patchy. According to Collins, the disease process mostly occupies the following sites: spine in 66% of patients, ribs in 45%, skull in 40%, shoulder in 40%, pelvis in 30%, long bones in 25%. The obtained aspirates can vary considerably with the percentage of clonal plasma cells and even with their presence or absence (56). Clonal plasma cells are also characterized by high membrane instability. The collection technique, the conditions and transport time determine the ability to objectively determine their percentage. Very important factors are the experience of the staff performing the reading and interpretation of the result. Certainly there are still many unrecognized factors which have an impact on measuring error. Many scientists are struggling to eliminate them in the future and check new diagnostic methods which might be less susceptible to mistakes e.g. ASO-RQ-PCR (57).

The group of patients characterized by the presence of discrepancies is not uniform, it encompasses four different types of inconsistencies. The presence of three of these is described in the available literature. A fourth group, characterized only by the presence of abnormal FLCs, concerned only four patients in the population analyzed by me. It might be assumed that the causes of the aforementioned inconsistencies may be: human error associated with the collection of material for research, conditions and time of transport, accuracy when applying the material to the camera, staff experience, freezing of the serum sample (shorter half-life compared to a complete immunoglobulin molecule FLC), errors of used diagnostic equipment, reagent lot changes. Often the cause of the observed discrepancies remains unknown (11).

CONCLUSIONS

At least several diagnostic tests are required in order to reliably assess the MM status before AHSCT and clonal plasma cell assessment by FC may remain only an additional diagnostic tool.

BIBLIOGRAPHY

1. Kyle RA, Rajkumar SV: Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia 2009; 23: 3-9.
2. Kyle RA, Steensma DP: History of multiple myeloma. Recent Results Cancer Res 2011; 183: 3-23.
3. Rajkumar SV: Treatment of multiple myeloma. Nat Rev Clin Oncol 2011; 8: 479-491.
4. Bhaskar A, Gupta R, Vishnubhatla S et al.: Angiopoietins as biomarker of disease activity and response to therapy in multiple myeloma. Leuk Lymphoma 2013; 54: 1473-1478.
5. Storti P, Bolzoni M, Donofrio G et al.: Hypoxia-inducible factor (HIF)-1α suppression in myeloma cells blocks tumoral growth *in vivo* inhibiting angiogenesis and bone destruction. Leukemia 2013; 27: 1697-1706.
6. Talamo G, Farooq U, Zangari M et al.: Beyond the CRAB symptoms: a study of presenting clinical manifestations of multiple myeloma. Clin Lymphoma Myeloma Leuk 2010; 10: 464-468.
7. Landgren O, Kyle RA, Pfeiffer RM et al.: Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood 2009; 113: 5412-5417.
8. Kumar S, Rajkumar SV: Will the real myeloma please stand up? Leukemia 2013; 27: 760-761.
9. Mikhael JR, Dingli D, Roy V et al.: Management of newly diagnosed symptomatic multiple myeloma: updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus guidelines 2013. Mayo Clin Proc 2013; 88: 360-376.
10. Katzmann JA, Snyder MR, Rajkumar SV et al.: Long-term biological variation of serum protein electrophoresis M-spike, urine M-spike, and monoclonal serum free light chain quantification: implications for monitoring monoclonal gammopathies. Clin Chem 2011; 57: 1687-1692.
11. Dispenzieri A, Kyle R, Merlini G et al.: International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia 2009; 23: 215-224.
12. Katzmann JA, Kyle RA, Benson J et al.: Screening panels for detection of monoclonal gammopathies. Clin Chem 2009; 55: 1517-1522.
13. Jenner E: Serum free light chains in clinical laboratory diagnostics. Clin Chim Acta 2014; 427: 15-20.
14. Bradwell AR, Carr-Smith HD, Mead GP et al.: Highly sensitive automated immunoassay for immunoglobulin free light chains in serum and urine. Clin Chem 2001; 47: 673-680.
15. Hobbs JAR: Modes of escape from therapeutic control in myelomatosis. Br Med J 1971; 5: 325.
16. Katzmann JA, Dispenzieri A: Screening algorithms for monoclonal gammopathies. Clin Chem 2008; 54: 1753-1755.
17. Jenkins MA: Serum and urine electrophoresis for detection and identification of monoclonal proteins. Clin Biochem Rev 2009; 30: 119-122.

18. Nowrousian MR, Brandhorst D, Sammet C et al.: Serum free light chain analysis and urine immunofixation electrophoresis in patients with multiple myeloma. Clin Cancer Res 2005;11: 8706-8714.
19. Katzmann JA: Screening panels for monoclonal gammopathies: time to change. Clin Biochem Rev 2009; 30: 105-111.
20. Bhole MV, Sadler R, Ramasamy K: Serum-free light-chain assay: clinical utility and limitations. Ann Clin Biochem 2014 Sep; 51(Pt 5): 528-542.
21. Singhal S, Vickrey E, Krishnamurthy J et al.: The relationship between the serum free light chain assay and serum immunofixation electrophoresis, and the definition of concordant and discordant free light chain ratios. Blood 2009; 2(114): 38-39.
22. Paiva B, Martinez-Lopez J, Vidriales MB et al.: Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. J Clin Oncol 2011; 29: 1627-1633.
23. Chae H, Cha K, Kim M et al.: Evaluation of the heavy/light-chain assay for the diagnosis and monitoring of multiple myeloma. Int J Lab Hematol 2013; 35: 10-12.
24. Solomon A: Light Chains of Human Immunoglobulins. Meth Enzymol 1985; 116: 101-121.
25. Mead GP, Reid S, Augustson B et al.: Correlation of Serum Free Light Chains and Bone Marrow Plasma Cell Infiltration in Multiple Myeloma. Blood 2004; 104: 4865, p299b.
26. Tang G, Snyder M, Rao LV: Assessement of serum free light chain (FLC) assays with immunofixation electrophoresis (IFE) and bone marrow (BM) immunophenotyping in the diagnosis of plasma cell disorders. Clin Chem 2008; 54: Abstr A-96.
27. Junghans RP, Anderson CL: The protection receptor for IgG catabolism in the ß-2-microglobulin containing neonatal intestinal transport receptor. Proc Natl Acad Sci USA 1996; 93: 5512-5516.
28. Russo L, Bakris GL, Comper WD: Renal handling of albumin: A critical Review of Basic Concepts and Perspective. Am J Kidney Dis 2002; 39: 899-919.
29. Alexanian R, Barlogie B, Dixon D: Renal failure in multiple myeloma; pathogenesis and prognostic implications. Arch Intern Med 1990; 150: 1693-1695.
30. Herrera GA, Joseph L, Gu X et al.: Renal pathologic spectrum in an autopsy series of patients with plasma cell dyscrasia. Arch Pathol Lab Med 2004; 128: 875-879.
31. Sanders PW, Booker BB, Bishop JB, Cheung HC: Mechanisms of intranephronal proteinaceous cast formation by low molecular weight proteins. J Clin Invest 1990; 85: 570-576.
32. Sethi S, Rajkumar SV: Monoclonal gammopathy-associated proliferative glomerulonephritis. Mayo Clin Proc 2013; 88: 1284-1293.
33. Hassan SB, Hanna MO: Urinary κ and λ immunoglobulin light chains in normoalbuminuric type 2 diabetes mellitus patients. J Clin Lab Anal 2011; 2: 229-232.
34. Solling K: Light chain polymerism in normal individuals in patients with severe proteinuria and in normal with inhibited tubular protein reabsorption by lysine. Scand J Clin Lab Invest 1980; 40: 129-134.
35. Cirit M, Uzüm A, Ozen P et al.: The value of serum immunoglobulin free light chain assessment in patients with monoclonal gammopathies and acute renal failure. Turk J Haematol 2012; 29: 385-391.
36. Park JW, Kim YK, Bae EH et al.: Combined analysis using extended renal reference range of serum free light chain ratio and serum protein electrophoresis improves the diagnostic accuracy of multiple myeloma in renal insufficiency. Clin Biochem 2012; 45: 740-744.
37. Takagi K, Kin K, Itoh Y et al.: Human alpha 1-microglobulin levels in various body fluids. J Clin Pathol 1980; 33: 786-791.
38. Bradwell AR: Serum Free Light Chain Analysis. 5th ed. Birmingham. The Binding Site 2008: 176-183.
39. Girón JA, Shah SL: *Helicobacter pylori* infection and light chain gammopathy. Clin Dev Immunol 2013; 348562.
40. De Larrea CF, Cibeira MT, Elena M et al.: Abnormal serum free light chain ratio in patients with multiple myeloma in complete remission has strong association with the presence of oligoclonal bands: implications for stringent complete remission definition. Blood 2009; 114: 4954-4956.
41. Bradwell AR: Serum Free Light Chain Analysis. 5th ed. Birmingham. The Binding Site 2008: 148-158.
42. Bradwell AR: Serum Free Light Chain Analysis. 5th ed. Birmingham. The Binding Site 2008: 215-222.
43. Koshy N, Ong MG, Nordberg ML et al.: Oligosecretory biclonal multiple myeloma. J La State Med Soc 2013; 165: 215, 217-218.
44. Katzmann JA, Clark RJ, Abraham RS et al.: Serum reference intervals and diagnostic ranges for the free κ and free λ immunoglobulin light chains. Relative sensitivity for detection of monoclonal light chains. Clin Chem 2002; 48: 1437-1444.
45. Lock RJ, Saleem R, Roberts EG et al.: A multicenter study comparing two methods for serum free light chain analysis. Ann Clin Biochem 2013; 50: 255-261.
46. Hoedemakers RM, Pruijt JF, Hol S et al.: Clinical comparison of new monoclonal antibody-based nephelometric assays for free light chain kappa and lambda to polyclonal antibody-based assays and immunofixation electrophoresis. Clin Chem Lab Med 2011; 50: 489-495.
47. Hansen CT, Münster AM, Nielsen L et al.: Clinical and preclinical validation of the serum free light chain assay: identification of the critical difference for optimized clinical use. Eur J Haematol 2012; 89: 458-468.
48. Bradwell AR: Serum Free Light Chain Analysis. 5th ed. Birmingham. The Binding Site 2008: 17-33.
49. Wood PB, Mc Elroy YG, Stone MJ: Comparison of serum immunofixation electrophoresis and free light chain assays in the detection of monoclonal gammopathies. Clin Lymphoma Myeloma Leuk 2010; 10: 278-280.
50. Weiss BM, Abadie J, Verma P et al.: A monoclonal gammopathy precedes multiple myeloma in most patients. Blood 2009; 113: 5418-5422.
51. Ludwig H, Milosavljevic D, Zojer N et al.: Immunoglobulin heavy/light chain ratios improve paraprotein detection and monitoring, identify residual disease and correlate with survival in multiple myeloma patients. Leukemia 2013; 27: 213-219.
52. Siti Sarah M, Nor Aini U, Nurismah MI et al.: Lambda light chain myeloma with co-migrating paraprotein at beta region on agarose gel electrophoresis: A case report. Clin Ter 2014; 165: 35-39.
53. Murray DL, Ryu E, Snyder MR, Katzmann JA: Quantitation of serum monoclonal proteins: relationship between agarose gel electrophoresis and immunonephelometry. Clin Chem 2009; 55: 1523-1529.
54. Paiva B, Vídriales MB, Rosiñol L et al.: A multiparameter flow cytometry immunophenotypic algorithm for the identification of newly diagnosed symptomatic myeloma with an MGUS-like signature and long-term disease control. Leukemia 2013; 27: 2056-2061.
55. Bradwell A, Harding S, Fourrier N et al.: Prognostic utility of intact immunoglobulin Ig'κ/Ig'λ ratios in multiple myeloma patients. Leukemia 2013; 27: 202-207.
56. Collins C: Multiple myeloma. [In:] Husband JE, Resnik RH (eds.): Imaging in Oncology. Vol. 2: 1998.
57. Puig N, Sarasquete ME, Balanzategui A et al.: Critical evaluation of ASO RQ-PCR for minimal residual disease evaluation in multiple myeloma. A comparative analysis with flow cytometry. Leukemia 2014; 28: 391-397.

received/otrzymano: 07.04.2015
accepted/zaakceptowano: 30.04.2015