International Staging System required standardization of biochemical laboratory testing in multiple myeloma

M. TICHÝ[1,13,14], V. MAISNAR[2,13], V. PALIČKA[1], B. FRIEDECKÝ[1], J. VÁVROVÁ[1], H. NOVOTNÁ[3], Z. ČERMÁKOVÁ[3], M. DASTYCH[3], P. ČECHÁK[4], D. VOGTOVÁ[4], E. JAROLÍMKOVÁ[5], H. BENÁKOVÁ[5], L. HACHOVÁ[5], D. BEZDÍČKOVÁ[5], F. KOUŘIL[6], E.A. ZÁBRANSKÁ[6], J. ŽENKOVÁ[7], P. SLABÝ[7], V. ŠČUDLA[8,13], E. GREGORA[9,13], I. ŠPIČKA[10,13], J. STRAUB[10,13], M. SCHÜTZOVA[11,13], R. HÁJEK[12,13]

[1]Institute of Clinical Biochemistry, e-mail: tichy@fnhk.cz, Charles University Hospital, 500 05 Hradec Králové, Czech Republic; [2]Department of Clinical Hematology, Charles University Hospital, Hradec Králové; [3]Department of Clinical Biochemistry and Hematology, University Hospital Brno; [4]Department of Biochemistry and Pathobiochemistry, Charles University, 3rd Faculty of Medicine, Prague; [5]Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University, Prague; [6]Department of Clinical Biochemistry, Faculty of Medicine, Palacky University, Olomouc; [7]Institute of Clinical Biochemistry and Hematology, Charles University, Faculty of Medicine, Pilsen; [8]3rd Department of Internal Medicine University Hospital Olomouc; [9]Department of Clinical Hematology Charles University, Prague; [10]1st Department of Internal Medicine, Charles University Hospital, Prague; [11]Department of Hematology/Oncology, Charles University Hospital, Pilsen; [12]Clinic of Internal Medicine-Hemato-Oncology, University Hospital Brno, Czech Republic; [13]Czech Myeloma Group; [14]Faculty of Military Health Sciences, Hradec Králové, University of Defence Brno, Czech Republic

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The standardization of biochemical measurement procedures in multiple myeloma is necessary for reliable prognostic stratification of patients in multicentric trials. The new prognostic index International Staging System for multiple myeloma uses only two laboratory markers, albumin and beta-2 microglobulin. Our study compared results of albumin, beta-2 microglobulin and monoclonal immunoglobulin measurements from six centers which provide treatment for multiple myeloma in the Czech Republic and attempted to standardize the analytic procedures. We have found that the measurement of albumin is well standardized and the results from all laboratories were comparable. The measurement of beta-2 microglobulin achieved comparability only after a partial unification of analytical methods. The determination of monoclonal immunoglobulin concentration provided comparable results for concentrations higher than 20 g/l with higher variability for lower values.

Key words: multiple myeloma, monoclonal immunoglobulin, albumin, beta-2 microglobulin, standardization

Multiple myeloma (MM) is the clinically most important monoclonal gammopathy. Prognosis of MM patients is very variable with median survival of 4 to 5 years. However, the survival for different subgroups of patients can range from 6 months to 10 years. This variability is due to the heterogeneous character of the disease as well as to individual variability between patients. The recently validated International Staging System (ISS) uses only two laboratory parameters, serum albumin and serum beta-2 microglobulin (B2M), to predict survival of MM patients [1, 2]. There are three stages of MM according to the ISS: I. B2M lower than 3.5 mg/l and albumin higher than 35 g/l (median survival for this stage is 62 months), II. B2M and albumin levels between stages I. and III. (median survival of 44 months), and III. B2M higher than 5.5 mg/l (median survival of 29 months) [1]. However, the published reports and guidelines do not specify the methodology for the measurement of these two parameters. We have studied interlaboratory variability in the measurements of these methods as well as monoclonal immunoglobulin and attempted to standardize the used analytical methods in order to prevent or minimize the impact of differences in laboratory methods on the management of patients within a multicentric therapeutic trial of Czech Myeloma Group.

Material and methods

Patients, samples and participating laboratories. The patients were enrolled in the CMG2002 multicentric clinical trial run by the Czech Myeloma Group and gave their informed consent to using their blood samples for research pur-

poses. All blood samples were taken and processed in a single center. Serum samples were then divided into six aliquots and frozen to –80 °C. The samples were transported to six different centers on dry ice. The time from taking a sample out of the freezer (–80 °C) to its handover was less than 3 hours. The temperature of samples at handover was not higher than –70 °C. Measurements were done on the same day in duplicate. Fibrin interference was prevented by centrifugation of the serum prior to the analysis. Six Czech laboratories participated in the study (General University Hospital, Prague; University Hospital, Prague – Vinohrady; Hradec Kralove University Hospital, Olomouc University Hospital, Brno University Hospital, and Pilsen University Hospital).

Albumin. The photometric method was used to measure serum albumin concetration. This method is based on the affinity of the acid-base indicator bromocresol green (BCG) to a specific binding site on the albumin molecule at pH 4.2 [3].

Beta-2 microglobulin. Different methods were used in the participating centers for the measurement of B2M in the first phase of the project, including radioimmunoassay (RIA), microparticle enzyme immunoassay (MEIA; Abbot AxSYM), luminoimmunoassay (LIA; Immulite 2000), and immunoturbidimetry (TURB; Olympus AU 2700). In the second phase of the project, RIA was abandoned because of incompatibility of its results with the other methods.

Monoclonal immunoglobulin. All samples contained only one paraprotein. The method used for the measurement of total protein in serum was based on the biuret reaction. The monoclonal immunoglobulin was subsequently determined quantitatively by serum protein electrophoresis (HYDRAGEL, Sebia and Capillarys, Sebia, France).

Results

The quantitation of serum albumin was highly reproducible in our study, with the 95% confidence interval of 1.3 to 3.0% and the coefficient of variability of 2.3 to 5.8%. These values are well within the current 9% tolerance limit for serum albumin measurement.

The results for B2M confirmed the initial suspicion that the B2M measurement can be substantially influenced by different analytical methods (Tab. 1). Results from RIA were significantly higher (in average about 55%) than results obtained using LIA, MEIA and immunoturbidimetry. This has led to the recommendation to abandon RIA for B2M measurement. In the second phase, the results for B2M testing have improved when all participating laboratories except one adopted MEIA, LIA and immunoturbidimetry methods. The results from the centers were comparable and the coefficient of variability was in all cases lower than 15.5%, a tolerance limit reflecting biologic variability of this method (Tab. 1).

The analysis of 12 samples of serum containing monoclonal immunoglobulin has shown that levels lower than 20 g/l were associated with higher variability (CV% = 22.1). Results for concentrations higher than 20 g/l were comparable between participating laboratories (CV% = 6.9), which is important for the diagnosis of MM and staging according to the DURIE and SALMON criteria [4] (Tab. 1, Fig. 1).

Table 1. Summary of results of measurements for biochemical prognostic markers in multiple myeloma and interlaboratory variability

Analyte	No. of samples	Mean concentration	Mean 95% confidence interval	SD	Median CV (%)
Albumin	11	43.10 g/l	40.48 – 45.66	1.4	3.1
b2-microglobulin I	11	3.60 mg/l	2.91 – 4.30	0.79	21.00
b2-microglobulin II	25	2.79 mg/l	2.50 – 3.08	0.36	12.70
Monoclonal Ig	12	20.77 g/l	19.17 – 22.36	2.04	11.45

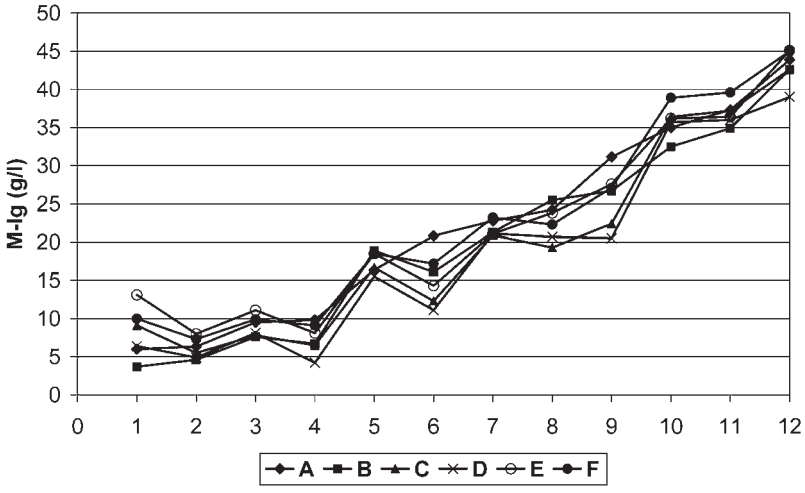

Figure 1. Concentrations of monoclonal immunoglobin in the serum of multiple myeloma patients as measured in different laboratories (A – University Hospital of Hradec Králové, B – General University Hospital of Prague, C – University Hospital of Prague-Vinohrady, D – University Hospital of Olomouc, E – University Hospital of Brno Bohunice, F – University Hospital of Pilsen).

Discussion

We have shown that there are considerable interlaboratory differences in the determination of basic biochemical prognostic parameters in MM, especially B2M. These potentially can lead to important inconsistencies in the staging

of patients in multicentric trials. The need for validation of laboratory testing is rarely recognized or addressed when such trials are designed or evaluated, especially when "routine" biochemical testing is involved. It is possible that the interlaboratory differences could partially explain the fact that the prognostic value of ISS is somewhat decreased in smaller multicentric trials, as has been shown on a cohort of 185 MM patients undergoing autologous stem cell transplantation within the 4W randomized clinical trial [5] as well as in a group of 270 patients treated by conventional chemotherapy [6]. In both cases, the ISS had only limited power to predict prognosis and especially the differences between stages I and II were blunted [5].

In our study, RIA was incompatible with other methods used for B2M measurement, including LIA, MEIA, and TURB. After abandoning RIA method, results from all six participating centers became comparable and the variability acceptable.

The results for serum albumin have confirmed that it is one of the most standardized analytical methods. Since 1994 when the certified reference material BCR CR M-470 was implemented, manufacturers of analytic tools have used it for the calibration of their products. This has led to comparability of serum albumin results worldwide [7].

It is difficult to standardize the measurement of monoclonal immunoglobulin due to several obstacles in the process, that include the errors in the quantitation of total serum protein, differences between various electrophoresis methods (agarose, acetylated cellulose, capillary electrophoresis), influence of instrumentation (e.g. the type of densitometer), the fact that the detection of monoclonal gradient is subjective and skill-dependent, and the overlay of the monoclonal gradient with other proteins in the zone [8]. Nevertheless, all the results reported from laboratories in this study were sufficiently comparable to be used in the clinic.

In conclusion, the standardization of biochemical methods for the determination of prognostic parameters in MM is both necessary and useful for the correct stratification of patients within multicentric therapeutic clinical trials as well as for the optimal utilization of the ISS prognostic index. However, the optimal albeit expensive solution for multicentric clinical trials would probably be the cryopreservation of samples with subsequent analysis in a single reference laboratory.

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