doi:10.4149/neo_2010_02_102

The importance of serum levels of selected biological parameters in the diagnosis, staging and prognosis of multiple myeloma

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Received July 7, 2009

The study aimed at evaluating the relation of 7 parameters associated with the internal biological properties of myeloma cells and the bone marrow microenvironment to multiple myeloma (MM) stages, distinguishing its initial/asymptomatic phase from monoclonal gammopathy of undetermined significance (MGUS) and assessing their relation to myeloma prognosis. In the studied group comprising 286 individuals (89 MGUS and 179 MM patients), statistically significant differences (Mann-Whitney test) between MGUS and MM at the time of diagnosis were found in the serum levels of HGF (hepatocyte growth factor), VEGF (vascular endothelial growth factor), ICTP (intercellular – carboxy-terminal telopeptide of type I collagen), PINP (procollagen type I N-terminal propeptide), OPG (osteoprotegerin) and syndecan-1/CD138, but not in Fas. Multivariate analysis (logistic regression) revealed an unsatisfactory potential of all the 7 studied indicators to discriminate between MGUS and MM. A deeper analysis showed statistically significant differences between MGUS and the initial/asymptomatic phase of MM (stage 1 according to the International Staging System) only in the cases of syndecan-1 (p=0.001) and Fas (p=0.008). The assessment of initial values of HGF, VEGF, ICTP, PINP, OPG, syndecan-1 and Fas showed a statistically significant relation (log rank test) to the overall survival (OS) in a group of 132 patients treated with conventional chemotherapy only in the cases of syndecan-1 (p=0.0002) and Fas (p=0.018), but in none of the investigated parameters in a group of 74 patients treated with HDT/ASCT (high-dose therapy/autologous stem cell transplantation). The analysis showed that, despite significant differences in serum levels of 6 of the 7 studied parameters found between MGUS and MM, none of the markers may be included in the spectrum of indicators used to distinguish the two conditions. Despite the positive relation, especially of syndecan-1 and, to a lesser extent, of Fas to the OS in patients treated with conventional chemotherapy, these prognostic factors are not applicable to HDT/ASCT.

Key words: multiple myeloma, monoclonal gammopathy of undetermined significance, angiogenic cytokines, bone metabolism, International Staging System, prognosis

Multiple myeloma (MM) is an unusually heterogeneous B-cell malignancy with individually different clinical course, characterized by uncontrolled proliferation and progressive accumulation of plasma cells, osteolytic bone disease, accompanying cytokine overproduction with the crucial role of bone marrow (BM) microenvironment dysregulation [1, 2]. Neovascularization and interactions between plasma cells and microenvironmental cells including osteoclasts, resulting in osteoclast activation, are two important processes contributing to the pathogenesis and clinical manifestation of MM [3].

The presented study aimed at evaluating the serum levels of 7 selected parameters with close relations to the internal biological properties of myeloma cells and to properties of the bone marrow microenvironment in MM, i.e. markers of angiogenesis (HGF – hepatocyte growth factor and VEGF – vascular endothelial growth factor), markers of bone turnover (ICTP – carboxy-terminal telopeptide of type I collagen, PINP – procollagen type I N-terminal propeptide and OPG – osteoprotegerin) and syndecan-1 (CD138), separately in monoclonal gammopathy of undetermined significance (MGUS) and various phases of MM progression (stages 1-3) according to the International Staging System (ISS) [4]. The reason for such an analysis is that the current IMWG (International Myeloma Working Group) criteria for diagnosing both MGUS and MM are descriptive, based on quantitative assessment of commonly available parameters, mostly express-

ing only the size of the tumor mass and ignoring markers showing the internal biological properties of myeloma tissue [5]. However, such a diagnostic system is unable to prevent potential diagnostic inaccuracy or treatment errors. Therefore, it is natural to attempt to reveal markers which would distinguish MGUS from MM more precisely and thus contribute to early detection of the onset of malignant transformation of MGUS. Some attention was also paid to assessing the role of the studied markers in MM prognosis and their relation to the overall survival (OS).

Patients and methods

The analyzed group of 268 subjects comprised 89 individuals meeting the IMWG diagnostic criteria for MGUS and 179 MM patients who met both the IMWG and SWOG (Southwest Oncology Group) criteria for myeloma, examined at the time of diagnosis before therapy was initiated [5, 6, 7]. The basic characteristics of both groups, i.e. MGUS and MM group are shown in Table 1. To analyze the overall survival, the set of MM patients was subdivided into groups treated with either conventional chemotherapy (CT) or high-dose therapy supported by autologous stem cell transplantation (HDT/ASCT). In the group of 132 CT-treated individuals, the median age was 68 (33-90) years and the M/F ratio was 0.85. Their distribution into ISS stages 1, 2 and 3 was as follows: 17%, 28% and 55%, respectively. The patients were treated with the MPT (melphalan, prednisone and thalidomide) or CTD (cyclophosphamide, thalidomide and dexamethasone) regimens, or with bortezomib and dexamethasone in progressive or relapsing forms of the disease [7]. The group treated with HDT/ASCT comprised 47 patients with the median age of 57 (29-64) years and the M/F ratio of 0.62. The distribution into ISS stages 1, 2 and 3 was as follows: 32%, 43% and 25%, respectively. Prior to HDT/ASCT, induction therapy was administered with the VAD (vincristine, adriamycin and dexamethasone) or CTD "junior" (cyclophosphamide, thalidomide and dexamethasone) regimens; in the cases of progressive or relapsing phases of the disease, bortezomib in combination with dexamethasone and even with melphalan or adriamycin; in some individuals, lenalidomide with dexamethasone [7]. Individuals who had not achieved at least very good partial remission were given thalidomide whereas the others received no maintenance therapy [7].

The serum levels of the soluble form of ICTP (0.3-6.0 μg/L) were measured by ELISA, Orion Diagnostica Espoo, Finland; PINP (normal level > 50 years of age 16.3-73.9 μg/L) was determined by the Cobas 6000 kit, Roche Diagnostics, and OPG (3.7-4.4 pmol/L) by the BioVendor GmbH ELISA kit. The serum levels of HGF (671-1992 pg/mL), VEGF (62-707 pg/mL), syndecan-1/CD138 (37-123 ng/mL) and Fas (4792-17150 pg/mL) were analysed by the quantitative sandwich enzyme immunoassay technique using the Quantikine kits, R&D Systems, Minneapolis. When compared with the HDT/ASCT group, the conventionally treated patients showed mostly higher serum levels of the 7 studied biological parameters: HGF (median 1772 vs. 1282 pg/mL), VEGF (233 vs. 275 pg/mL), ICTP (9.7 vs. 5.2 μg/L), PINP (60 vs. 48 μg/L), OPG (6.3 vs. 5.4 pmol/L), syndecan-1/CD138 (219 vs. 88 ng/mL) and Fas (7976 vs. 6685 pg/mL). The cut-off values for distribution within the individual groups required for the prognostic analysis (determination of OS) were set at the upper limits of normal ranges. The exceptions were Fas, with the discrimination value equal to the median of the obtained values, and syndecan-1, with the upper limit respecting the limits of the calibration curve used. The statistical analysis was performed using the Pearson's χ^2 and non-parametric Mann-Whitney U tests with the p-value <0.05. For multivariate analysis, logistic regression was used. Overall survival curves for each parameter were calculated by the Kaplan-Meier method and compared using the log rank test ($p<0.05$).

Table 1. The basic characteristics of the analysed groups

	MGUS	MM
n	89	179
Age median (years)	62 (32-82)	64 (29-90)
M/F ratio	0.85	0.79
MIg type		
IgG	62 (70%)	118 (65.5%)
IgA	14 (16%)	37 (21%)
IgM	7 (8%)	-
Biclonal	3 (3.0%)	2 (1%)
Bence-Jones	3 (3.0%)	21 (12%)
IgD	-	1 (0.5%)
κ/λ ratio	1.2	1.7
MM stage (ISS)		
1	-	31 (17%)
2	-	50 (28%)
3	-	97 (55%)

MGUS – monoclonal gammopathy of undetermined significance, MM – multiple myloma, MIg – monoclonal immunoglobulin, ISS – International Staging System

Results

The analysis suggests that, in comparison with MGUS patients, those with MM have statistically very significantly higher levels of HGF, ICTP, OPG and syndecan-1 and, to a lesser extent, also the levels of PINP. Lower serum VEGF concentrations were of borderline significance whereas no statistically significant difference was noted in the Fas molecule (Table 2). The frequency of pathologically increased serum levels in the MM and MGUS groups are compared in Fig. 1. The analysis of serum levels of biological parameters in individual stages of MM according to ISS showed that serum concentrations increase naturally with more advanced stages of the disease (i.e. 1-3) in all stages in the cases of HGF, ICTP, OPG and syndecan-1, and between stages 2 and 3 in PINP. On the other hand, serum VEGF concentrations decreased

Table 2. Comparison of serum levels of selected biological parameters between a group of multiple myeloma patients examined at diagnosis and a group of individuals with monoclonal gammopathy of undetermined significance (n=268)

	MM		MGUS		
	n	**median (range)**	**n**	**median (range)**	**MM vs. MGUS ($p < 0.05$)**
HGF (pg/mL)	177	1672.0 (492 – 8000)	86	1004.0 (402 – 3834)	< 0.00001
VEGF (pg/mL)	176	237.3 (5.0 – 1990.0)	85	319.0 (3.8 – 1969)	0.04
ICTP (μg/L)	172	8.5 (1.8 – 616.0)	89	4.9 (1.0 – 33.1)	< 0.0001
PINP (μg/L)	171	53.8 (11.4 – 575.9)	89	45.0 (7.8 – 164.0)	0.001
OPG (pmol/L)	146	6.0 (1.2 – 60.0)	76	4.5 (0.4 – 16.6)	0.00001
Syndecan-1 (ng/mL)	172	189.1 (2.5 – 256.0)	78	42.2 (2.5 – 256.0)	< 0.00001
Fas (pg/mL)	139	7685.0 (1179 – 46655)	57	7431.0 (4858 – 89954)	NS

MM – multiple myeloma, MGUS – monoclonal gammopathy of undetermined significance, n – number, HGF – hepatocyte growth factor, VEGF – vascular endotelial growth factor, ICTP – carboxy-terminal telopeptide of type I collagen, PINP – procollagen type I N-terminal propeptide, OPG – osteoprotegerin

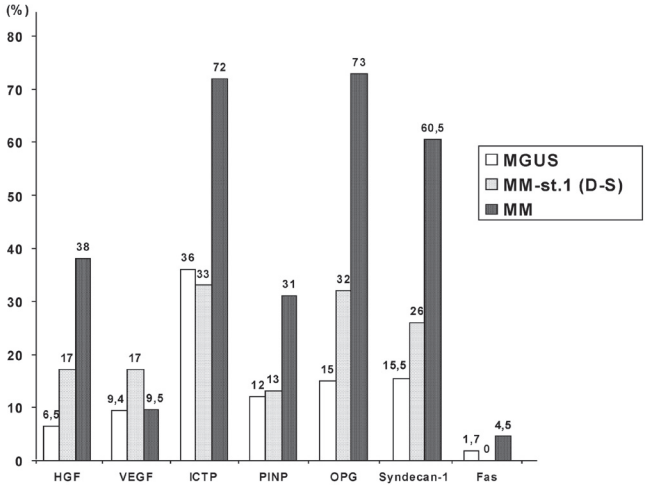

Fig. 1. Comparison of frequencies of abnormal levels of the studied biological parameters in MGUS, MM and stage 1 (according to the Durie-Salmon staging system) [6,7].

MGUS – monoclonal gammopathy of undetermined significance, MM – multiple myeloma, D-S – Durie-Salmon staging system, HGF – hepatocyte growth factor, VEGF – vascular endothelial growth factor, ICTP – carboxy-terminal telopeptide of type I collagen, PINP – procollagen type I N-terminal propeptide, OPG – osteoprotegerin.

between stages 1 and 3 (Table. 3). The comparison of serum levels of the studied parameters in stage 1 with those in MGUS showed statistically significantly higher levels in stage 1 only in the case of syndecan-1, but not in HGF, VEGF, ICTP and PINP. The MGUS group had statistically insignificantly higher levels of OPG and significantly higher levels of Fas when compared with stage 1 MM (Table 3). The comparison of frequency of abnormal levels of the studied parameters in the stage 1 MM and MGUS patients revealed that abnormal levels of HGF, VEGF, PINP, OPG and syndecan-1, but not ICTP and Fas molecules, were more frequent in stage 1 MM (Fig. 1). When the stage 2 MM and MGUS groups were compared, HGF and syndecan-1 showed statistically very significant differences while ICTP and OPG showed less significant differences; no differences were found in VEGF, PINP and Fas. When comparing the stage 3 MM and MGUS patients, very significant differences were those in HGF, ICTP, PINP, OPG and syndecan-1 and less significant in the cases of VEGF and Fas (Table 3). Logistic regression performed in the group of 179 MM patients showed that of the 7 analyzed parameters, only PINP, HGF and syndecan-1/CD138 have a certain potential to discriminate between MGUS and MM. It was found that a 10-μg/L rise of the PINP level means a 1.3-fold increase in probability of MM against MGUS (by 34%, with a rather wide confidence interval of 7-68%), a 100-pg/mL rise of the HGF level means a 1.2-fold increase in the probability (16%, CI=6-27%), and a 10-ng/mL rise of syndecan-1level means a 1.2-fold increase (15%, CI=7-23%).

The prognostic analysis of the group of 132 CT-treated MM patients revealed a statistically significant relationship between the initial serum levels and overall survival (OS) only in syndecan-1 and Fas (Table 4). The positive relationship between syndecan-1 and Fas serum concentrations and prognosis of patients treated with CT is confirmed by the shape of survival curves and duration of median survival (49 vs. 18 months and 49 vs. 25 months) (Figs. 2-C and 2-E). The analysis of 47 patients treated with HDT/ASCT did not show a statistically significant relationship to overall survival in any of the 7 investigated biological parameters, with the only exception being borderline significance in syndecan-1 (Table 4), also documented by markedly more favourable shape of the survival curve after 25 months from diagnosis with low levels of syndecan-1 and median OS (immeasurable OS vs. 21 months) (Fig. 2-D). It can be seen that, in the HDT/ASCT-treated patients, the Fas molecule lost its potential relation to prognosis as noted in the CT group (median OS 21 vs. 17 months). Also worth mentioning are the completely different

Table 3. Comparison of serum levels of selected biological parameters between patients with monoclonal gammopathy of undetermined significance and those with individual clinical stages of multiple myeloma according to the International Staging System [4]

		HGF (pg/mL)	VEGF (pg/mL)	ICTP (µg/L)	PINP (µg/L)	OPG (pmol/L)	Syndecan-1 (ng/mL)	Fas (pg/mL)
MM (ISS)								
Stage 1	**n**	31	31	31	31	28	31	21
	median (range)	1169.0 (498-8000)	340.8 (5.0-923.8)	4.1 (1.7-14.2)	49.9 (22.0-133.3)	3.7 (1.2-60.0)	95.1 (2.5-256.0)	6013 (4048-46655)
Stage 2	**n**	49	50	48	48	41	49	38
	median (range)	1383.0 (492-8000)	244.8 (34.5-1990.0)	7.1 (2.9-31.6)	49.5 (16.0-575.9)	5.7 (1.5-15.6)	134.0 (10.9-256.0)	7,576 (1179-13217)
Stage 3	**n**	96	94	92	91	76	91	79
	median (range)	2199.5 (535-8000)	203.7 (46.8-1679.3)	13.0 (3.1-616.0)	63.0 (11.4-348.9)	7.1 (2.2-56.6)	256.0 (8.0-256.0)	8,381 (2653-20000)
MGUS								
	n	87	86	89	89	77	79	58
	median (range)	1005.0 (402-3834)	319.1 (38-1969)	4.9 (1.0-33.1)	45.0 (7.8-164.0)	4.5 (0.4-16.6)	42.9 (2.5-256.0)	7,417 (3241-89954)
MGUS vs.	**Stage 1**	NS	NS	NS	NS	NS	0.001	0.008
MGUS vs.	**Stage 2**	0.0002	NS	0.004	NS	0.001	<0.00001	NS
MGUS vs.	**Stage 3**	<0.00001	0.008	<0.0001	<0.0001	<0.00001	<0.00001	0.04

MM – multiple myeloma, ISS – International Staging System, MGUS – monoclonal gammopathy of undetermined significance, NS – nonsignificant, HGF – hepatocyte growth factor,VEGF – vascular endothelial growth factor, ICTP – carboxy-terminal telopeptide of typ I collagen, PINP – procollagen type I N-terminal propeptide, OPG – osteoprotegerin

shapes of survival curves in HDT/ASCT patients in the case of HGF, with median OS 47 vs. 17 months but no statistical significance (Fig. 2-B, Table 4). Different, albeit statistically unconfirmed, trends for favourable prognosis were observed in the HDT/ASCT group in the cases of VEGF, ICTP, PINP and OPG, also supported by relative variations in median OS (Table 4).

Discussion

A serious problem in clinical practice is the lack of a marker with distinguishing ability between two very different conditions, MGUS and the initial/asymptomatic phase of MM. While MGUS is a benign or premalignant discrete clonal expansion of the plasma cell population, MM is a progressive, incurable and fatal disease. In pathobiology of MM, the key role is played by the imbalance between proliferation and apoptosis of monoclonal plasma cells, resulting in the expansion of tumor cells [8]. In addition to the internal properties of myeloma plasma cell, the BM microenvironment characteristics influencing the cytokinetic properties of plasma cells are also of crucial importance [2].

The presented analysis confirmed that practically all the studied soluble biological markers are related to the stages of myeloma progression and their levels are, with various statistical significance, different from those in MGUS. It is well known that MGUS is distinguished from MM by density of the capillary network in the bone marrow, caused by an overproduction of proangiogenic cytokines such as HGF, VEGF and bFGF (basic fibroblast growth factor) by clonal plasma cells [3, 9, 10], which stimulate vasculogenic differentiation of CD34+ cells as well as both proliferation and migration of endothelial cells [11, 12]. HGF, a pleiotropic cytokine, promotes proliferation and dissemination of myeloma cells in the BM and contributes to the development of myeloma bone disease by osteoblast inhibition [13]. Previous studies were inconsistent in terms of the relationship between serum HGF and VEGF levels and MM stage and prognosis, as some showed the relationship [3, 9, 14] but others did not [12]. Similarly ambiguous results were obtained when comparing MGUS and MM. In our studied group, serum HGF levels were increased in 38% of MM patients, similar to 43% in a previous study [15]. Higher serum HGF levels were found in only 6.5% of individuals with MGUS, with statistically significant difference being limited to advanced MM, i.e. stages 2 and 3. Therefore, it is not a suitable marker to distinguish between the initial/asymptomatic form of MM and MGUS. VEGF, a highly potent proangiogenic peptide is involved not only in acceleration of angiogenesis but also in the progression of MM, development of myeloma bone disease and resistance to therapy [12, 16]. In the presented analysis, the VEGF levels were surprisingly higher in MGUS than in advanced MM (stages 2 and 3), with the levels unexpectedly decreasing with the degree of MM progression (stages 1-3) but statistically significant differences only between MGUS and stage 3. Lower VEGF levels in stages 2 and 3 when compared with

Table 4. Results of prognostic analysis with respect to the overall survival in 7 selected biological markers in a group of 132 multiple myeloma patients treated with conventional therapy and in 47 patients treated with high-dose therapy with autologous stem cell transplantation

Parameter	Therapy	Cut off	n	(%)	Median overall survival (months)	Sig. (p<0.05)
HGF (pg/mL)	**CT**	< 1992	84	(64)	45	0.64
		≥ 1992	47	(36)	34	
	HDT/ASCT	< 1992	26	(56)	47	0.11
		≥ 1992	20	(44)	17	
VEGF (pg/mL)	**CT**	< 237	65	(50.5)	45	0.39
		≥ 237	64	(49.5)	45	
	HDT/ASCT	< 237	23	(49)	21	0.64
		≥ 237	24	(51)	47	
ICTP (μg/L)	**CT**	< 6	25	(20)	45	0.45
		≥ 6	102	(80)	45	
	HDT/ASCT	< 6	26	(58)	30	0.83
		≥ 6	19	(42)	21	
PINP (μg/L)	**CT**	< 74	83	(66)	45	0.97
		≥ 74	43	(34)	45	
	HDT/ASCT	< 74	38	(84)	21	0.41
		≥ 74	7	(16)	x	
OPG (pmol/L)	**CT**	< 6.4	61	(58)	x	0.164
		≥ 6.4	44	(42)	47	
	HDT/ASCT	< 6.4	21	(51)	28	0.88
		≥ 6.4	20	(49)	21	
Syndecan-1 (ng/mL)	**CT**	< 256	70	(55)	49	0.0002
		≥ 256	57	(45)	18	
	HDT/ASCT	< 256	30	(67)	x	0.05
		≥ 256	15	(33)	21	
Fas (pg/mL)	**CT**	< 7656	59	(56)	49	0.018
		≥ 7656	47	(44)	25	
	HDT/ASCT	< 7656	10	(30)	21	0.54
		≥ 7656	23	(70)	17	

n – number, Sig. – statistical significance, CT – conventional therapy, HDT/ASCT – high-dose therapy/autologous stem cell transplantation, HGF – hepatocyte growth factor, VEGF – vascular endothelial growth factor, ICTP – carboxy-terminal telopeptide of type I collagen, PINP – procollagen type I N-terminal propeptide, OPG – osteoprotegerin

stage 1 and no differences between individuals with MGUS and stage 1 MM were reported only by Sezer [12], but not by previous articles [14, 16]. In addition to the actual lack of difference between the two conditions compared, the potential cause may be the type of analytical method used and possible artificial release of VEGF from platelets during coagulation [17]. The above-mentioned facts suggest that determination of serum VEGF does not contribute to distinguishing between the asymptomatic phase of MM and MGUS.

Already the definitions of MGUS and MM indicate that the key criterion for distinguishing between the two conditions are manifestations of myeloma bone disease (MBD) reflecting impaired functional homeostasis of osteoclasts and osteoblasts resulting from dysbalance of the RANKL/OPG (receptor activator of nuclear factor -κB ligand) axis [18, 19, 20]. Myeloma cells induce overexpression of RANKL with decreasing availability of OPG in the BM microenvironment, resulting in enhanced osteoclastogenesis. Therefore, in the initial phase, osteogenic markers levels change before skeletal involvement is detected by MRI or FDG-PET/CT. The differences in bone resorption in MGUS and MM are characterized by ICTP, a highly sensitive indicator of osteoclastic bone resorption and prediction of very early myeloma bone lesions [19, 21, 22], related to bone pain intensity, number and severity of bone lesions and pathological fractures [20, 21, 23, 24]. The presented study found a statistically significant difference in ICTP levels between MM and MGUS, with increased ICTP levels in 3/4 of MM patients as well as in 1/3 of those with MGUS. Although previous studies also showed a close relationship of ICTP levels to the degree of MM progression [21, 23], our analysis revealed a lack of statistically significant difference between MGUS and stage 1 MM and thus impossible practical use of this marker to distinguish the two conditions. Consistently with sporadic previous studies, the evaluation of serum PINP, i.e. an indicator of osteoblastic activity and new bone formation, was rather ambiguous [21, 23]. The presence of different serum concentrations was similar in both MGUS and MM (12% vs. 31%), consistently with previous findings [21]. Although the serum

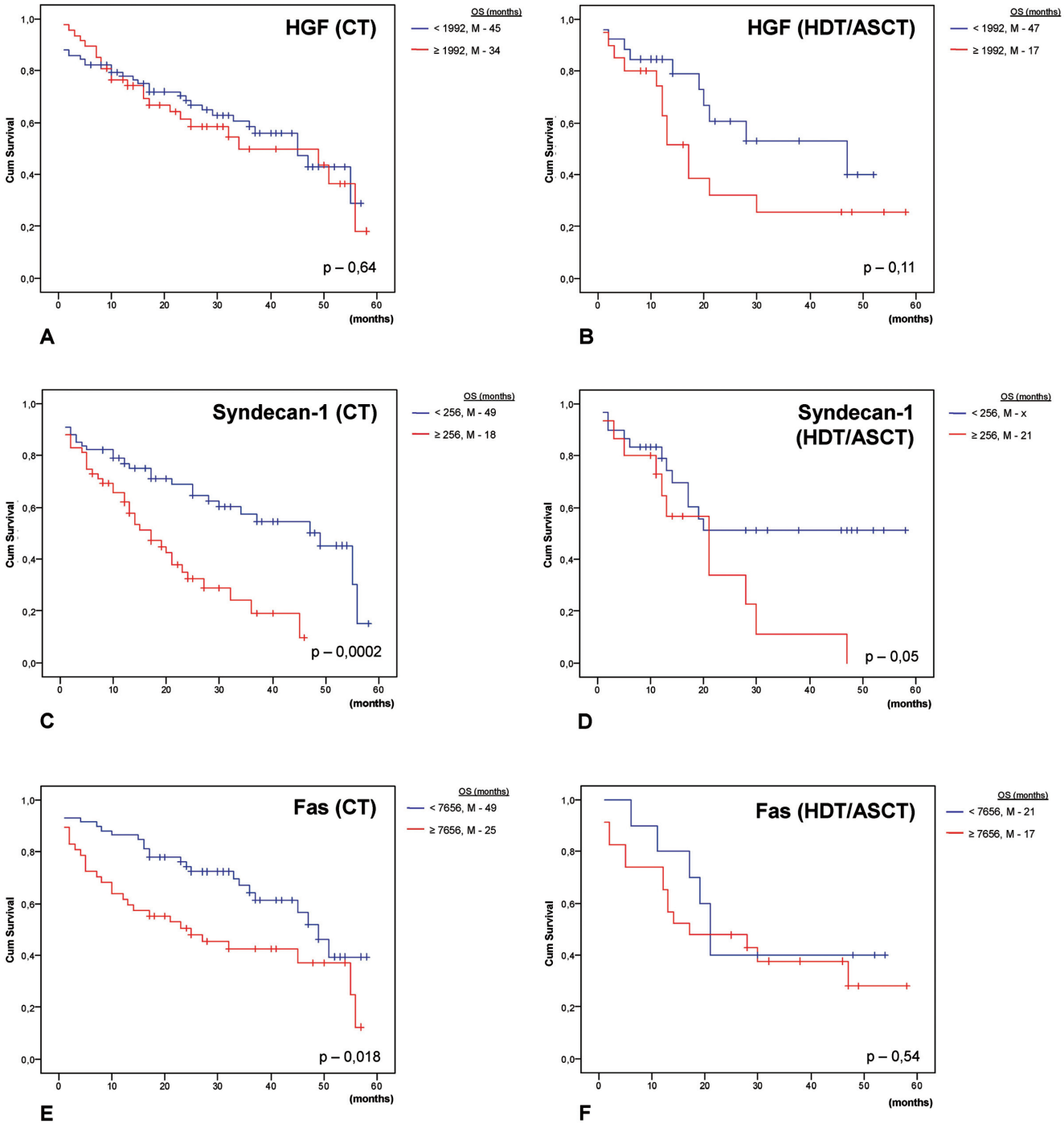

Fig. 2. Kaplan-Meier survival curves in a group of 132 patients treated with conventional chemotherapy (CT) and in a group of 47 patients treated with high-dose therapy supported by autologous stem cell transplantation (HDT/ASCT):

A – HGF (hepatocyte growth factor), CT-treated group; B – HGF, HDT/ASCT-treated group; C – syndecan-1, CT-treated group; D – syndecan-1, HDT/ASCT-treated group; E – Fas, CT-treated group; F – Fas, HDT/ASCT-treated group.

OS – overall survival, M – median overall survival.

PINP levels in MGUS and the entire group of MM patients were statistically different, the nearly identical serum levels in MGUS and stage 1 MM lacking statistical difference make the marker useless for distinguishing the two conditions. OPG,

acting as a decoy receptor antagonist for RANKL, is secreted by osteoblasts and stromal elements. The progression of MBD is associated with increased production of RANKL in the BM microenvironment and lower OPG production, resulting in decreased number and activity of osteoblasts and lower serum levels of OPG in MM when compared with a group of healthy individuals [25]. Some analyses suggested that serum OPG levels are decreased in MM [26, 27], which is in accordance with the immunohistochemical detection of decreased expression of OPG in osteoblasts of bone trabeculae [18]. However, studies have been published which show higher OPG levels in both MGUS and MM when compared with control groups, with no statistically significant differences between MGUS and MM [28]. Although in our study the frequencies of increased OPG levels in MGUS and MM were substantially different (15% vs. 73%), the difference in serum concentrations between MM and MGUS was only limited to stage 2 and, especially, stage 3. Very similar serum OPG levels in individuals with MGUS and asymptomatic MM (stage 1) make this biological marker useless for differentiation of the two conditions. One reason may be the fact that serum OPG levels do not reliably reflect the activity of OPG in the BM microenvironment, since there are other sources of serum OPG, including the capillary network where OPG acts as an antiapoptotic factor in endothelial cells. Syndecan-1(CD138), a transmembrane glycoprotein released from the surface of neoplastic plasma cells is one of the key regulatory substances playing a role in the pathobiology of MM by participating in the binding of extracellular matrix components, modulation of numerous representatives of the cytokine network and influencing the behaviour of myeloma cells as well as stimulation of osteoblast function and osteoclast inhibition [29, 30, 31]. It is a marker of viable myeloma plasma cells (CD38+ CD45- CD56+), the expression of which is rapidly lost during apoptosis. Previous studies showed a relationship between increased serum syndecan-1 levels to β2 -microglobulin levels, serum M-component levels as well as the extent of BM infiltration with myeloma plasma cells and disease activity [30, 31, 32, 33]. The frequency of increased serum syndecan-1 in MM patients is reported to range from 35% to 79% [29, 30, 33]; it was 60.5% in our group of patients. However, the close relationship between syndecan-1 levels and disease progression (stages 2 and 3), also declared in some earlier studies [29, 30, 32, 34], was not confirmed by other authors [31, 35]. What we consider important is detection of significant differences in serum syndecan-1 levels between MGUS and all three MM stages (1–3). Nevertheless, even syndecan-1 cannot be regarded as a suitable marker for distinguishing MGUS and the initial/asymptomatic phase of MM due to significantly overlapping serum concentrations in individual patients with MGUS and stage 1 MM. This correlates with findings from other studies considering syndecan-1 an excellent marker of plasma cell which does not contribute to distinguishing MGUS from MM [35, 36]. Fas (Apo-1/CD95) is a transmembrane receptor involved in the induction of cell apoptosis in the case of cross-linking with Fas ligand [37]. As most myeloma cells express the proapoptotic Fas antigen on their surface, its impaired function may contribute to MM progression [37]. It was found that soluble Fas antigen is involved in protecting myeloma cells from apoptosis and thus stimulates MM progression. Despite the underlying rationale, the presented analysis did not reveal differences in serum Fas levels between MGUS and MM, since in most individuals, the serum levels were within the normal range. It is of interest that in stage 1, serum Fas levels were even statistically significantly lower than in MGUS individuals. Multivariate logistic regression in fact confirmed the findings from separate analyses as even the three most suitable indicators (PINP, HGF and syndecan-1) of the 7 analyzed parameters were found to have only very low potential to discriminate between MGUS and asymptomatic MM, which makes the parameters practically useless.

The presented study suggest that of the 7 analyzed biological parameters, only two – syndecan-1 and Fas – showed a relationship to the OS of MM patients. Similar to previous studies [29, 30, 31, 32], it found a statistically significant positive correlation between different serum syndecan-1 (CD138) levels to OS in the group treated with conventional chemotherapy, with only marginal relationship to prognosis (OS) if HDT/ASCT was applied. The relation to overall survival was also found in initial Fas molecule levels, but only in the group of CT patients and not in the HDT/ASCT group. In the cases of VEGF, ICTP, PINP and OPG, prognostic analysis showed no statistically significant relation to survival in both CT and HDT/ASCT patients, although the shapes of survival curves and median OS were markedly different, especially in HGF when HDT/ASCT was applied.

In conclusion, the presented study suggests that, with the exception of the Fas molecule, there is a statistically significant differences in serum levels of all the analyzed indicators between MGUS and MM and, in the cases of HGF, ICTP, OPG, syndecan-1 and Fas, an increase in their serum concentrations with higher clinical stages of MM. With the only exception of serum syndecan-1/CD138 levels, no other readily available indicator was found to extend the current possibilities for distinguishing MGUS from the initial/asymptomatic phase of MM (stage 1). Prognostic analysis showed a significant relation of serum levels to OS only in syndecan-1 and Fas when standard conventional therapy was used but not in HDT/ASCT-treated patients. Therefore, it is clear that with currently dominating HDT/ASCT and especially intensive and tailored therapy with combinations involving modern immunomodulatory drugs (thalidomide, bortezomid, lenalidomide), it is essential to rapidly focus on the introduction of modern, highly sophisticated methods, i.e. molecular biology techniques, gene expression analysis, immunophenotypic analysis of myeloma cells or proteomics into standard clinical practice.

Acknowledgement. The study was supported by the grant NR 9500-3 and NR 9489-3 of the IGA of the Ministry Health of the Czech Republic and MSM 6198959205.

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