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Analysis of the serum levels of selected biological parameters in monoclonal gammopathy of undetermined significance and different stages of multiple myeloma

V. SCUDLA[1*], P. PETROVA[2], J. MINARIK[1], T. PIKA[1], J. BACOVSKY[1]

[1]*Department of Internal Medicine III;* [2]*Department of Clinical Biochemistry, University Hospital, Faculty of Medicine and Dentistry, Palacky University, Olomouc, 775 20, Czech Republic*

**Correspondence: vlastimil.scudla@fnol.cz*

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The aim of the study was to analyze differences in the serum levels of 8 selected biological parameters between monoclonal gammopathy of undetermined significance (MGUS) and different stages of multiple myeloma (MM), potentially beneficial for distinguishing between the two conditions. The analyzed group of 131 subjects comprised 62 individuals with MGUS and 69 MM patients examined at the time of diagnosis. The serum levels were determined by a quantitative immunoradiometric assay (insulin-like growth factor 1, IGF-1) and quantitative sandwich enzyme immunoassay (osteopontin, OPN; endostatin, ES; macrophage inflammatory proteins 1α/β, MIP-1α/β; angiogenin, ANG; and interleukin 17, IL-17). The analysis showed a statistically significant difference in serum concentrations between MGUS and the symptomatic form of MM using the Durie-Salmon (D-S) staging system only in the cases of OPN and stages II and III (0.001 and < 0.0001), osteocalcin (OC) and stage II (0.006) and MIP-1α and stage 3 (0.0002). With the International Staging System (ISS), significant differences were found between MGUS and the individual stages of MM in the cases of OPN and stages I-III (0.003, 0.001 and < 0.0001, respectively); ES and stages II and III (0.015 and 0.00009, respectively); MIP-1α and stage III (0.00001), and ANG and stage II (0.014). Unlike in MGUS, the serum levels in impaired renal function (D-S substage B) were significantly increased in OC (0.011), OPN (0.003), ES (0.0001), MIP-1α (0.0004), ANG (0.005) and IGF-1 (0.014), but only in OPN (< 0.0001) in the case of substage A. A small proportion of MM patients was characterized by substantially raised levels of OC (13%), OPN (16%) and ES (11%), exceeding the highest concentrations detected in MGUS (> 49.9 ng/mL, 288 ng/mL and 328.4 ng/mL, respectively). The unsatisfactory potential of the studied parameters to distinguish MGUS from MM, with a partial exception of OPN, results from the fact that the parameters are secondary and do not reflect the principal differences in the biology of MGUS and MM. More benefit may be expected from analyses using multiparametric immunophenotyping of plasma cells and molecular biology methods including gene expression analysis and proteomics.

Key words: monoclonal gammopathy of undetermined significance, multiple myeloma, clinical stages, biological markers, osteopontin

The differences between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) have received much attention recently, as illustrated by both the concept of multistep pathogenesis of MM with confirmed transition of all MM cases from MGUS and the establishment of International Myeloma Working Group (IMWG) criteria for the diagnosis of both conditions as a starting point for adequate treatment [1,2,3,4]. These discrimination criteria, based on the quantitative assessment of parameters expressing the load of plasma cell population ignore markers expressing intrinsic biological properties of plasma cells and the bone marrow (BM) microenvironment. Therefore, such a system is incomplete and does not reflect state of the art knowledge of pathobiology of MGUS and MM and cannot reliably distinguish MGUS from MM, especially in the initial/asymptomatic phase.

A previous study analysing a group of 268 individuals with MGUS and MM found significant differences in the serum levels of β_2 microglobulin, thymidine kinase, HGF, ICTP, OPG and syndecan 1/CD138 and less significant differences in VCAM-1, PINP and vascular endothelial growth factor (VEGF). A deeper analysis showed statistically significant differences between MGUS and the initial/asymptomatic form of MM only in the case of syndecan 1 [5,6]. Another analysis revealed significant differences between MGUS and MM in the serum levels of

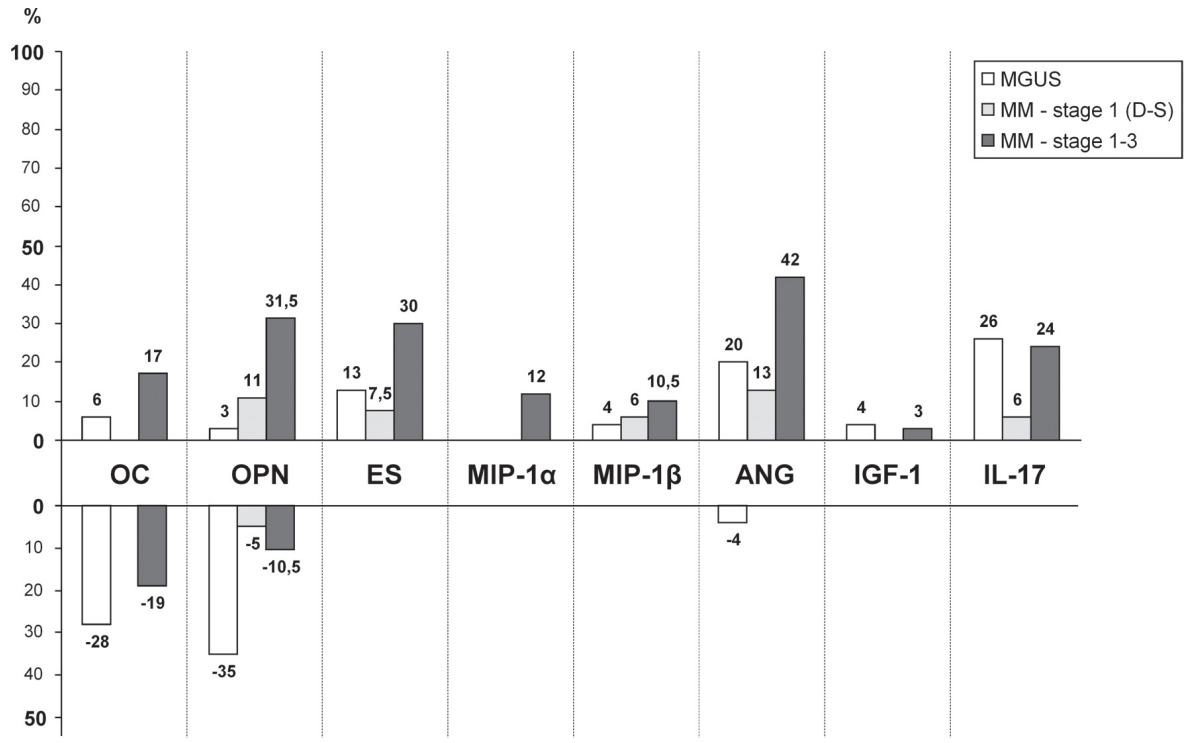

Figure 1. Graphical comparison of the frequency of increased levels of the analyzed parameters in individuals with monoclonal gammopathy of undetermined significance (MGUS) and those in different stages of multiple myeloma as assessed by the Durie-Salmon (D-S) staging system at the time of diagnoses prior to therapy.
OC – osteocalcin, OPN – osteopontin, ES – endostatin, MIP-1α/β – macrophage inflammatory proteins 1α and β, ANG – angiogenin, IGF-1 – insulin-like growth factor 1, IL-17 – interleukin 17

Table 1. The basic characteristics of the analyzed groups

		MGUS	MM
n		62	69
Age median (years)		67 (31-84)	68 (32-86)
M/F ratio		0.55	1.1
MIg type	**IgG**	46 (74%)	41 (60%)
	IgA	8 (13%)	12 (17%)
	IgM	6 (10%)	
	Bence-Jones	-	12 (17%)
	Nonsecretory	-	2 (3%)
	IgD	1 (1.5%)	1 (1.5%)
	Biclonal	1 (1.5%)	1 (1.5%)
	κ/λ ratio	1.3	2.2
MM stage (D-S)			
	1		6 (9%)
	2		26 (37%)
	3		37 (54%)
Substage	**A**		58 (84%)
	B		11 (16%)
MM stage (ISS)			
	1		25 (36%)
	2		23 (33%)
	3		21 (31%)

MGUS – monoclonal gammopathy of undetermined significance, MM – multiple myeloma, MIg – monoclonal immunoglobulin, D-S – Durie-Salmon staging system, ISS – International Staging System

thymidine kinase, ICTP, MIP-1α, osteopontin, HGF, syndecan 1 and the κ/λ light chain ratio and, to a lesser extent, in the cases of angiogenin and endostatin [7]. The presented study aimed at assessing the differences between MGUS and individual stages of MM progression using the Durie-Salmon (D-S) staging system [8] and International Staging System (ISS) [9] for biological parameters shown by *in vitro* studies and experimental models to play a role in the pathogenesis of MGUS and MM that have not been paid enough attention in the literature, that is osteocalcin (OS), osteopontin (OPN), endostatin (ES), macrophage inflammatory proteins 1α and β (MIP-1α, MIP-1β), angiogenin (ANG), insulin-like growth factor 1 (IGF-1) and interleukin 17 (IL-17) [10,11,12,13,14,15,16,17,18,19].

Patients and methods

The analyzed group of 138 subjects comprised 69 individuals meeting the IMWG diagnostic criteria for MGUS and 69 MM patients who met both the IMWG and Southwest Oncology Group (SWOG) criteria for MM, examined at the time of diagnosis before initiation of therapy [3,8]. To avoid bias, seven patients were excluded from the original group as according to the current criteria, it was impossible to distinguish individuals with transition of MGUS with

those in the asymptomatic phase (stage I) of MM. The basic characteristics of both MGUS and MM groups are shown in Table 1.

The serum levels of OC (> 50 years 14-46 ng/mL) were determined by the Cobas 6000 diagnostic kit (Roche Diagnostics). The levels of IGF-1 (< 269 ng/mL) were measured by an immunoradiometric assay manufactured by Immunotech, Prague, Czech Republic. The serum levels of OPN (49.2-175.0 ng/mL), ES (58-232 ng/mL), MIP-1α (< 46.9 pg/mL), MIP-1β (< 212 pg/mL), ANG (19000-437000 pg/mL) and IL-17 (< 31.2 pg/mL) were analyzed by the quantitative sandwich enzyme immunoassay technique using the Quantikine kits (R&D Systems, Minneapolis, USA). All measurements were performed in duplicate according to the manufacturer's instructions and were reproducible. The statistical analysis was performed using the Pearson's χ^2 test, non-parametric Mann-Whitney U test and, if needed, paired Student's t-test ($p < 0.05$).

Results

The analysis suggested that MGUS patients tend to have normal or even decreased levels of the studied parameters, when compared with those with newly diagnosed MM. The frequency of different, mostly increased, levels found in MM patients, as compared with the normal ranges in those with MGUS, is shown in Figure 1. None of the analyzed parameters can be reliably used to distinguish MGUS from the initial/asymptomatic phase of MM (D-S stage IA) just by the mere presence of abnormal values.

The analysis revealed both subnormal and increased levels of serum OC in MGUS as well as MM. When using the D-S staging system, OC levels below the normal range were found in 28% of MGUS patients, none of stage I MM but 19% of MM patients (15% stage II and 25% stage III). Increased OC levels were observed in only 6% of MGUS patients but 17% of those with MM (0% stage I, 25% stage II and 13% stage III). The levels of OPN were decreased in 35% and elevated in only 3% of MGUS individuals. This is in a sharp contrast to MM patients, with lower OPN concentrations found in 10.5% (34% stage I, 17% stage II and 15% stage III) and increased serum levels in 31.5% (16% stage I, 25% stage II and 49% stage III) of them. When assessing parameters related to angiogenesis, increased ES levels were less frequent in MGUS patients (13%) than in MM patients (30%; 20% stage I, 21% stage II and 37.5% stage III). Serum ANG levels were increased in 20% and decreased in 4% of individuals with MGUS, and increased in 42% of patients with MM (40% stage I, 39% stage II and 49% stage III). As seen in Figure 1, the frequency of increased serum levels of IGF-1, MIP-1α, MIP-1β and IL-17 was rather low, with no significant differences between the MGUS and MM groups. Statistical analysis comparing the levels in MGUS with those in individual MM stages revealed increasing differences in some of the parameters, depending on the degree of disease progression. When comparing MGUS with the initial/asymptomatic phase of MM (D-S stage IA), a statistically significant difference was not found in any of the studied parameters. The comparison of MGUS and stage II MM showed significant differences in OC and OPN levels and the comparison with stage III significant

Table 2. Comparison of serum levels of selected biological parameters between patients with monoclonal gammopathy of undetermined significance and those with different clinical stages of multiple myeloma according to the Durie-Salmon staging system (8)

MM (D-S)		OC (ng/mL)	OPN (ng/mL)	ES (ng/mL)	MIP-1α (pg/mL)	MIP-1β (pg/mL)	ANG (pg/mL)	IGF-1 (ng/mL)	IL-17 (pg/mL)
Stage 1	n	5	6	5	5	5	5	5	5
	median	18.9	65.6	151.9	10	115.9	369034	120	15
	(range)	(14.5-29.4)	(35.7-183)	(101.1-271.6)	(10-11.5)	(34-539)	(275338-451540)	(97-218)	(15-50)
Stage 2	n	21	25	20	21	20	19	20	21
	median	34	103	181.2	10.9	80.4	381600	147	15
	(range)	(9.6-105.5)	(26.7-500)	(86.1-332.9)	(10-1500)	(11-750.7)	(195140-648494)	(78-204)	(15-105)
Stage 3	n	32	34	31	30	30	29	30	31
	median	20.3	162.5	197.4	20.1	82.2	405600	144.5	15
	(range)	(7-128.9)	(3-500)	(73.5-500)	(10-155.3)	(11-257.4)	(190852-800000)	(17-284)	(15-101)
MGUS	n	15	61	55	54	55	55	53	54
	median	19.9	56.5	154.4	10	86.9	348160	131	15
	(range)	(4-49.9)	(10.5-288)	(70.9-328.4)	(10-37.4)	(11-354)	(121980-682668)	(25-305)	(15-106)
MGUS vs.	stage 1	NS	NS	NS	NS	NS	NS	NS	NS
MGUS vs.	stage 2	0.006	0.0001	NS	NS	NS	NS	NS	NS
MGUS vs.	stage 3	NS	<0.0001	NS	0.0002	NS	NS	NS	NS

MM – multiple myeloma, D-S – Durie-Salmon staging system, OC – osteocalcin, OPN – osteopontin, ES – endostatin, MIP-1α/β – macrophage inflammatory protein 1α/β, ANG – angiogenin, IGF-1 – insulin-like growth factor 1, IL-17 – interleukin 17, MGUS – monoclonal gammopathy of undetermined significance, NS – nonsignifiant difference

differences in OPN and MIP-1α levels, with no differences found in the cases of ES, MIP-1β, ANG, IGF-1 and IL-17 (Table 2). It must be considered, however, that the number of D-S stage I patients was low, so the results of statistical analysis must be taken with caution. The comparison of MGUS and MM substage A or B (substage B: creatinine level > 170 μmol/L) showed that only serum levels of OPN were significantly higher in substage A (Table 3). There were more differences between MGUS and substage B, with statistically significant differences in the serum levels of OC, OPN, ES, MIP-1α , ANG and IGF-1 (Table 3).

Analysis based on the ISS prognostic stratification of MM and comparing MGUS with the initial phase of MM (stage I) showed significantly higher levels only in the case of OPN (Table 4). When MGUS was compared with stage II MM, the serum levels of OPN, ES and ANG were significantly higher, and when it was compared with stage III, significantly higher serum levels were noted in OPN, ES and MIP-1α. No differences between MGUS and MM stages I-III were found in the serum levels of OC, MIP-1β, IGF-1 and 1L-17. A more detailed analysis showed that the serum levels of OC > 49.9 ng/mL,

Table 3. Comparison of serum levels of selected biological parameters between patients with monoclonal gammopathy of undetermined significance and those with different clinical substages A vs. B of multiple myeloma according to the Durie-Salmon staging system (8)

MM (D-S)		OC (ng/mL)	OPN (ng/mL)	ES (ng/mL)	MIP-1α (pg/mL)	MIP-1β (pg/mL)	ANG (pg/mL)	IGF-1 (ng/mL)	IL-17 (pg/mL)
Substage A	n	48	56	47	47	47	45	46	48
	median	23.1	120	165.8	11.5	86.5	376560	129	15
	(range)	(7-128.9)	(3-500)	(73.5-500)	(10-1500)	(11-750.7)	(190852-800000)	(17-284)	(15-105)
Substage B	n	10	9	9	9	8	8	9	9
	median	37.8	289.2	315.6	22.9	66.7	459832	176	27
	(range)	(12-105.5)	(39.7-500)	(189.6-397.6)	(10-155.3)	(32.8-257.4)	(368920-632200)	(110-201)	15-93
MGUS	n	15	61	55	54	55	55	53	54
	median	19.9	56.5	154.4	10	86.9	348160	131	15
	(range)	(4-49.9)	(10.5-288)	(70.9-328.4)	(10-37.4)	(11-354)	(121980-682668)	(25-305)	(15-106)
MGUS vs.	substage A	NS	<0.0001	NS	NS	NS	NS	NS	NS
MGUS vs.	substage B	0.011	0.003	0.0001	0.0004	NS	0.005	0.014	NS

MM – multiple myeloma, D-S – Durie-Salmon staging system, OC – osteocalcin, OPN – osteopontin, ES – endostatin, MIP-1α/β – macrophage inflammatory protein 1α/β, ANG – angiogenin, IGF-1 – insulin-like growth factor 1, IL-17 – interleukin 17, MGUS – monoclonal gammopathy of undetermined significance, NS – nonsignifiant difference

Table 4. Comparison of serum levels of selected biological parameters between patients with monoclonal gammopathy of undetermined significance and those with different clinical stages of multiple myeloma according to the International Staging System (9)

MM (ISS)		OC (ng/mL)	OPN (ng/mL)	ES (ng/mL)	MIP-1α (pg/mL)	MIP-1β (pg/mL)	ANG (pg/mL)	IGF-1 (ng/mL)	IL-17 (pg/mL)
Stage 1	n	22	25	22	22	22	21	22	22
	median	24.8	95.5	145.4	10	90.6	347584	137.5	15
	(range)	(8.3-88.9)	(26.7-500)	(73.5-271.6)	(10-25.5)	(34-539)	(190852-524816)	(78-218)	(15-105)
Stage 2	n	18	22	17	18	17	17	17	18
	median	191	120	218.3	15,3	58.4	406094	129	15
	(range)	(9.6-86.6)	(3-406.4)	(97.2-500)	(10-105.4)	(11-257.4)	(238218-800000)	(81-201)	(15-93)
Stage 3	n	18	18	17	16	16	15	16	17
	median	32.1	185.6	249.6	24.8	72.4	427186	149	15
	(range)	(7-128.9)	(45.7-500)	(125.1-397.5)	(10-1500)	(32.8-750.7)	(243400-632200)	(17-284)	(15-15)
MGUS	n	15	61	55	54	55	55	53	54
	median	19.9	56.5	154.4	10	86.9	348160	131	15
	(range)	(4-49.9)	(10.5-288)	(70.9-328.4)	(10-37.4)	(11-354)	(121980-682668)	(25-305)	(15-106)
MGUS vs.	stage 1	NS	0.003	NS	NS	NS	NS	NS	NS
MGUS vs.	stage 2	NS	0.001	0.015	NS	NS	0.014	NS	NS
MGUS vs.	stage 3	NS	<0.0001	0.00009	0.00001	NS	NS	NS	NS

MM – multiple myeloma, ISS – International Staging System, OC – osteocalcin, OPN – osteopontin, ES – endostatin, MIP-1α/β – macrophage inflammatory protein 1α/β, ANG – angiogenin, IGF-1 – insulin-like growth factor 1, IL-17 – interleukin 17, MGUS – monoclonal gammopathy of undetermined significance, NS – nonsignifiant difference

OPN > 288 ng/mL and ES > 328.4 ng/mL are highly suggestive of the diagnosis of MM since serum concentrations exceeding those limits were not detected in the MGUS group (Tables 2-4). However, this conclusion is reliable only for small numbers of MM patients (13% in the case of OC, 16% in OPN and 11% in ES) which makes practical use rather limited.

Discussion

Practically all MM cases were shown to be linked to earlier MGUS. However, no universal marker that would reliably distinguish the two entities with such different prognoses and therapeutic approaches is currently available in clinical practice [4,6,20,23]. In the pathobiology of the transition from MGUS to MM, a key role is played by nuclear factor kappa B (NF-κB) involved in the development of MM, including osteoclastic bone resorption prevailing over osteoblast formation and the degree of neoangiogenesis, that is processes directly linked to the course of clinical picture and prognosis of MM [21,22]. Apart from high or increasing levels of M-protein with highly pathological κ/λ free light chain ratio, the commonly used yet not completely reliable predictive factors for the transition from MGUS to MM are decreasing haemoglobin levels, a higher count and different morphology of monotypic plasma cells in BM including their detection in peripheral blood and detection of their abnormal immunophenotype and increased proliferative activity, detection of increased bone remodelling documented by an increase in bone turnover markers and pathologic picture of whole-body MRI and FDG-PET/CT assessment of the skeleton [20,23,24]. Despite some advances, molecular mechanisms involved in the transformation of MGUS to MM remain largely unexplained. Therefore, even current clinical practice is based on long-term monitoring and detection of clinical and laboratory progression [1,20,23,25].

Assessment of the serum levels of OC in MM has yielded ambiguous results. OC is produced by osteoblasts (OBLs). Decreased OBL activity in MM as compared with both MGUS and healthy individuals is accompanied by low serum levels of OC whereas increased concentrations in stage I MM correlate with larger resorption and osteoid areas [26,27,28]. By contrast, OC levels are increased in D-S stage I MM as compared with stages II and III [29]. Whereas in advanced MM, OC levels are decreased due to suppressed bone formation, they are normal or raised in the initial phase with only mild skeletal involvement [10,11,27,30,31,32]. Given the late decrease of bone formation in MM, OC is not considered a suitable marker for distinguishing MGUS from MM, since serum OC levels in patients with the initial/asymptomatic form of MM (stage I) are similar to those in MGUS, as confirmed by this study [11]. Moreover, detailed analysis showed a significant difference in the OC levels between MGUS and stage II MM and more frequent subnormal OC levels in MGUS as compared with MM. Significantly higher levels of OC in our group of patients with substage B resulted from increased serum levels within an already small decrease in glomerular filtration [16]. The inability of OC to reliably distinguish MGUS from MM is, among other facts, due to the focal character of osteolytic lesion in MM, with natural bone formation potentially continuing in unaffected parts of the skeleton, and/or due to the fact that potential skeletal microfractures are accompanied by local stimulation of OBLs [16].

OPN is a multifunctional cytokine with a broad spectrum of biological effects, including those on angiogenesis, immune processes and bone homeostasis, especially in osteoclastogenesis and the development of myeloma bone disease. It is expressed and secreted by myeloma cells, osteoclasts (OCLs), OBLs and other elements of the bone marrow environment, with OPN secretion being correlated with the degree of expression of the *maf* transcription factor [12,33]. It is involved in the processes of adhesion, chemotaxis, migration, stimulation of proliferation and inhibition of apoptosis of tumor cells, and in regulation of cell signalling pathways in neoplastic transformation. It is considered to be a mediator of tumor growth and progression [34]. The degree of OPN expression is related to NF-κB activation and, together with IL-6, it plays a key role in the growth and survival of myeloma cells [33,35]. Immunocytochemical analysis showed significant OPN positivity in myeloma plasma cells in advanced MM but not in plasma cells in MGUS. Cultures of advanced MM plasma cells produced more OPN than cultures of plasma cells collected from patients with smouldering MM and MGUS. Serum levels of OPN were significantly higher in MM patients than in those with MGUS. Increased serum OPN levels were also noted in transition from MGUS to MM [36,37]. It has been ascertained that high titres of OPN in serum do not always correlate with the degree of malignant progression and severity of skeletal involvement. Therefore, the prognostic potential of OPN must always be assessed together with the results of *maf* subtyping, producing a biologically different entity [33]. Our study revealed significant differences in the serum levels of OPN in individual MM stages. Significant differences in OPN levels were found by the D-S and ISS staging systems between MGUS and stages II and III MM but only by the ISS between MGUS and stage I MM. This study did not confirm differences in OPN levels between MGUS and the initial/asymptomatic phase of MM as reported by an earlier study, which may be due to a low proportion of D-S stage I patients [36]. The difference in serum levels between MGUS and MM is illustrated by only 3% of increased OPN levels in MGUS as compared with 31.5% in MM, with the levels rising with higher MM stages, using both stratification systems. By contrast, lower-than-normal OPN levels were observed in 35% of MGUS patients and only 10.5% of those with MM. Significantly higher OPN levels in patients with impaired renal function were reflected by statistically significant difference between substages A and B (based on the D-S system). A certain differential diagnostic potential of OPN to distinguish MGUS and MM is illustrated by the finding that the OPN level exceeded 288 ng/mL in none of the MGUS patients but in 16% of those with MM.

When compared with MM, MGUS has much lower density of the capillary network in BM. Whereas MGUS is a "preangiogenic" condition, MM is "vascular", with the increase in angiogenesis being a key mechanism in MM pathogenesis [21,38]. ES is an endogenous inhibitor of angiogenesis involved in maintaining angiogenic balance. It interacts with endothelial cell molecules and leads to their apoptosis by inhibiting anti-apoptotic proteins bcl-2 and bcl-XI [13,39]. It is considered a negative biomarker for tumor progression since inhibition of neoangiogenesis and stabilization of the vessel wall results in suppression of tumor growth [13]. Serum ES levels were insignificantly lower in the MGUS group than in stages II and III MM but almost identical in the initial/asymptomatic phase of MM. Similarly, the frequency of increased ES levels was substantially lower in MGUS than in MM (13% vs. 30%). In 11% of MM patients they exceeded the highest level found in MGUS, that is 328.4 ng/mL. MIP-1α and MIP-1β are pluripotent chemokines playing an important role in the pathogenesis and clinical picture of MM [10,11,40]. They are involved, among other things, in OCL differentiation and thus the development and severity of myeloma bone disease, and also lead to suppression of immunoglobulin synthesis [15,32,40,41]. The biological effects of MIP-1α are mediated by fibroblast growth factor receptor 3 (FGFR3) and activation of the RAS-MAPK signalling pathway that modulates proliferation and survival of myeloma cells and thus the speed of tumor growth and patients' overall survival [14]. Expression of MIP-1α in myeloma cells was shown to be high in 59%, moderate in 13% and missing in 28% of MM patients, with a good correlation with both serum levels and the density of BM capillary network [42]. In accordance with the fact that MIP-1α expression was undetected in plasma cells in MGUS, no MGUS patients were found to have increased serum levels whereas in stage III MM (both D-S and ISS), MIP-1α concentrations were significantly higher, being 2-fold higher in substage B [16]. The assessment of MIP-1β levels proved to be of no value as the increase was observed in only a very small number of patients and no differences were found between MGUS and individual MM stages. ANG, a polypeptide with angiogenic activity is involved in the processes of tumor growth and bone healing. It is expressed in vascular endothelium, fibroblasts, lymphocytes and tumor elements. During neovascularization, it is involved in degradation of membrane laminin and fibronectin, resulting in disruption of the vascular wall and subsequent migration of endothelial cells. In MM, increased levels of ANG, VEGF and angiopoietin were detected, with a significant drop after therapy [16]. In the MGUS group, ANG was increased in 20% of MGUS individuals and 42% of MM patients. The levels were very similar, with the only differences between MGUS and stage II (according to the ISS) as well as substage B. IGF-1, a paracrine growth factor for myeloma cells, induces their proliferation, inhibition of apoptosis, invasion and migration, as well as affinity to the BM microenvironment elements [17,18]. The IGF-1/IGF-1R interaction plays an important role in the pathogenesis of MM, with the IGF-1 effect being mediated by activation of the ras-MAPK and P13K/Akt-1 signalling cascade with persistent activation of NF-κB [18]. A pilot study showed significantly lower IGF-1 levels in MGUS and MM as compared with healthy individuals, with the levels being substantially lower in MM than in MGUS [43]. In our study, however, IGF-1 levels were only sporadically higher in MGUS than in MM, with significantly higher levels in substage B. Thus, the assessment of serum IGF-1 levels in MGUS and MM is not beneficial. IL-17, a disulphide-linked homodimer produced by activated memory T cells is considered a mediator between the immune and hematopoietic systems. It stimulates the production of IL-6, ICAM and other parameters in the BM environment elements. The production of IL-17 in the BM of patients with MM T-helper lymphocytes is mediated by dendritic cells. Unlike in MGUS, the BM of MM patients contains a high proportion of Th-17-1 cells with coexpression of 1L-17 and IFN-γ, interacting with apoptotic myeloma elements [19]. In spite of these relations, the presented analysis revealed no differences in the serum levels between MGUS and MM.

In conclusion, the results suggest that the attempt to modify the IMWG criteria by adding markers that would make the diagnosis of MGUS and especially the initial/asymptomatic phase of MM failed. One of the reasons may be the fact that between MGUS and the initial phase of MM, there is a smooth line of transitional grades with various rates of neoplastic transformation. Of the 8 analyzed markers, only OPN levels were substantially different between MGUS and the initial phase of MM. For distinguishing MGUS from MM, there was a certain benefit from the detection of high levels of OC (> 49.9 ng/mL), OPN (> 288 ng/mL) and/or ES (> 328.4 ng/mL), that is rates exceeding the highest detected serum concentration in the MGUS group. However, this was only true for a small number of patients (13%, 16% and 11%, respectively). The real role of OPN, MIP-1α and ES in the pathobiology of MM was confirmed by a significant gradual increase in their concentrations from MGUS to the most advanced stages of MM. Another valuable finding was the detection of significantly higher levels of OPN, ES, MIP-1α, ANG and IGF-1 in substage B. The study showed that assessing the serum levels of analytically easily available biological markers is not beneficial for distinguishing MGUS from the initial/asymptomatic phase of MM since these markers are secondary and do not reflect the principal differences in the pathobiology of MGUS and MM. More promising is the use of multiparametric immunophenotyping of plasma cells and new molecular biology methods including gene expression analysis and proteomics.

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