IVYSPRING INTERNATIONAL PUBLISHER

Journal of Cancer

2014; 5(7): 518-524. doi: 10.7150/jca.9266

Research Paper

HGF, sIL-6R and TGF-β_1 Play a Significant Role in the Progression of Multiple Myeloma

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Received: 2014.03.31; Accepted: 2014.05.22; Published: 2014.06.11

Abstract

Background. In the last few years, it has been widely reported that proinflammatory and angiogenic cytokines are important for the development and progression of multiple myeloma (MM).

Objectives. To further validate and acquire more insight into this view we decided to check whether plasma levels of certain cytokines and their soluble receptors differ between MM patients and healthy subjects.

Patients and Methods. The study was conducted in 76 MM patients aged 22 to 77 years (60±10 years) and 35 healthy controls aged 20 to 63 years (33±10 years). Plasma levels of interleukin-6 (IL-6), b-fibroblast growth factor (b-FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and transforming growth factor-β_1 (TGF-β_1), as well as soluble receptors for IL-6 (sIL-6R) and VEGF (sVEGF-R2) were measured using enzyme-linked immunosorbent assay (ELISA).

Results. Significantly higher plasma levels of IL-6 (13.65±42.61 *vs.* 1.04±1.12 pg/ml, p=0.006), HGF (2174±2714 *vs.* 648±130 pg/ml, p<0.001), b-FGF (7.92±10.78 *vs.* 2.54±5.38 pg/ml, p<0.001) and sIL-6R (37.1±14.2 *vs.* 25.3±6.4 ng/ml, p=0.003) were observed in MM patients *vs.* healthy controls, respectively. Plasma sVEGF-R2 was significantly lower in MM patients than in controls (7518±2119 *vs.* 8725±1281 pg/ml, respectively; p<0.001). We observed an inverse correlation between length of treatment and the level of sIL-6R, and TGF-β_1 in plasma.

Conclusions. Plasma levels of HGF, b-FGF, IL-6 and sIL-6R in MM patients were higher when compared to the control group. Antineoplastic therapy leads to a time-dependent decrease in plasma levels of sIL-6R, and TGF-β_1 in MM patients. Blood plasma level of HGF is an optimal measure to differentiate patients in whom disease is progressing versus patients who respond to therapy.

Key words: b-FGF, cytokines, HGF, interleukin-6, multiple myeloma.

Introduction

Recent studies have confirmed the importance of soluble cytokines and their receptors in the biology of multiple myeloma (MM) [1-3]. The principal mechanism of action of interleukin-6 (IL-6) in MM is most likely the inhibition of apoptosis of myeloma cells. It has been shown that the level of soluble receptors for IL-6 (sIL-6R) is elevated in myeloma patients compared to healthy subjects, and its level correlates with

disease severity [3,4]. Currently, the serum level of sIL-6R is considered to be one of the prognostic factors in MM [4]. Vascular endothelial growth factor (VEGF) is one of the strongest drivers of angiogenesis. In MM, the binding of VEGF with its receptors, VEGF-R1 and VEGF-R2, induces angiogenesis and leads to exponential growth of the tumour. Furthermore, with the activation of these receptors, there is increased expression of integrins on the cell surface, which facilitates adhesion and migration [6, 7, 8]. In MM, the serum level of basic fibroblast growth factor (b-FGF) increases with disease progression. It was also found that myeloma cells express surface receptor FGF-R3 (which is not found on normal lymphoid cells), divide more rapidly and undergo apoptosis less frequently than cells without FGF-R3. Effective antiproliferative treatment often causes a reduction in b-FGF levels in the serum of patients with MM [1,9,10]. Transforming growth factor-β_1 (TGF-β_1) is synthesized by almost all cells of the body. The increased microvascular network in the bone marrow of patients with MM is likely the result of the plasma secretion of TGF-β_1, which stimulates the release of platelet derived growth factor and thereby enhances angiogenesis [11]. Hepatocyte growth factor (HGF) has mitogenic, chemotactic, and morphogenic properties. It has a significant effect on the morphogenesis of new blood vessels and bone marrow stroma, and it stimulates the synthesis of angiogenic factors. Elevated levels of HGF in the serum of patients with MM is associated with an unfavourable prognosis. Numerous studies have shown that HGF is involved in the progression of MM, including damage to bone, angiogenesis, and myeloma cell adhesion to bone marrow stroma [12,13]. Studies indicate the importance of the HGF-c-MET axis in the stimulation of proliferation and inhibition of apoptosis in MM cells [4,15,16].

The aim of this study was to compare the plasma levels of cytokines IL-6, VEGF, b-FGF, TGF-β_1, HGF, and soluble receptors sIL-6R and sVEGF-R2 between patients with MM and healthy volunteers.

Patients and methods

Clinical characteristics of the study group

The study comprised 76 patients aged 22 to 77 years (mean age 60 years, SD ± 10) with MM treated at the Department of Haematology, University Hospital, Krakow, Poland. The control group consisted of 35 healthy volunteers aged 20 to 63 years (mean age 33 years, SD ± 10). Both test and control groups were similar with respect to gender distribution. The group of patients with MM included 38 women and 38 men, F/M ratio = 1. The control group consisted of 19 women (54.3%) and 16 men (45.7%) F/M ratio = 1.2. The diagnosis of MM was based on cytologic examination of bone marrow aspirate revealing at least 10% plasmocytes, the presence of monoclonal proteins in serum or urine, and bone osteolytic lesions. Patients with MM were divided into 2 groups, designated N for newly diagnosed, previously untreated, and S for patients undergoing antiproliferative treatment. Group N consisted of 29 patients (38.2%), while group S consisted of 47 people (61.8%). We subdivided group S into three groups, based on length of treatment: S1 included patients undergoing treatment for 1 to 2 years (n=13), S2 comprised patients undergoing treatment for 2 to 3 years (n=11), and S3 consisted of patients undergoing treatment for longer than 3 years (n=23). Additionally, any patient in group S undergoing treatment for more than a year was evaluated for response to antiproliferative therapy. Patients with MM were treated according to the guidelines of the Polish Myeloma Group. For patients under 65 years of age, first line treatment consisted of the CTD protocol (cyclophosphamide, thalidomide, and dexamethasone) followed by CTX and G-CSF combined with the auto-PBSCT procedure. Throughout treatment we continued thalidomide. In cases of disease recurrence, treatment consisted of bortezomib or lenalidomide in many cases. Patients over 65 years of age were treated either with the MPT (melphalan, prednisone, and thalidomide) or VMP (bortezomib, melphalan and prednisone) protocols. In cases of disease progression, lenalidomide and dexamethasone were given [17]. We evaluated whether there was progression of disease, resistance to treatment, or if treatment had a positive effect. These analyses were based on: assessment of bone marrow (the percentage of plasma cells in bone marrow biopsy), protein parameters (serum protein electrophoresis), biochemical parameters (serum C-reactive protein - CRP, β2 microglobulin, calcium), and complete blood count. During our testing we were unable to evaluate the clinical outcome of treatment in 6 patients, and therefore testing was conducted on the remaining group of 41 patients. Of these remaining patients 23 positively responded to treatment while 18 patients showed progressive MM which was resistant to therapy. The study was approved by the local Ethics Committee.

Blood and plasma analysis

Blood samples were collected between 7:30 and 8:30 hours in sterile Vacutainer tubes (Becton Dickinson, New Jersey, USA) which contained an anticoagulant (EDTA). The tubes were then centrifuged for 10 minutes at 1,000 RPM. Plasma was pipetted into sterile tubes (Nunc, Roskilde, Denmark) and stored at -70°C until analysis was performed. Biochemical as-

says of lactate dehydrogenase (LDH), blood urea, creatinine, total calcium concentration in both groups and CRP, β_2 microglobulins, serum protein electrophoresis in MM group were performed using standard methods. Results of blood counts were obtained via photo-optical and conductivity methods using a Cell-Dyn 1700 analyzer (Abbott, Abbott Park, USA). The plasma levels of VEGF, sVEGF-R2, IL-6, sIL-6R, b-FGF, HGF, and TGF-β_1 were measured using ELISA assays (Quantikine, R&D Systems, Minneapolis, MN., USA).

Statistical methods

Results were subjected to statistical analysis. The normality of the distribution was tested using the Shapiro-Wilk test. We used Student's t-test for the analysis of independent variables, and the U Mann-Whitney test for the analysis of dependent variables. All calculations were performed using STATISTICA 10 PL software (StatSoft, Inc., USA) licensed to Jagiellonian University. Only results which had a p value <0.05 were considered statistically significant.

Result

Plasma levels of cytokines, their soluble receptors, and the assessment of biochemical parameters in patients with MM versus the control group are presented (Tables 1 and 2). Elevations of HGF, b-FGF, IL-6, and sIL-6R were observed in the blood plasma of patients with MM as compared to the control group, whereas the plasma level of sVEGF-R2 was lower compared to the control group. Analyses revealed differences in the clinical values of erythrocytes, haemoglobin, haematocrit, platelet count, urea, creatinine, and LDH activity in serum between MM patients and controls. We also analyzed concentrations of cytokines and their soluble receptors in MM patients paying attention to differences that may be attributed to age and sex. The only differences noted between the sexes was the concentration of b-FGF, which was twice as high in males when compared with women (Table 3). We did not, however, observe any statistically significant differences attributed to age (Additional file 1: supplementary Table 1). We compared the levels of cytokines and soluble cytokine receptors, depending on the application and duration of disease treatment. Higher levels of TGF-β1 were observed in group N patients when compared to group S (Table 4). We further analyzed patients depending on duration of therapy (Table 5). During initial therapy, mean plasma IL-6 levels decreased three fold compared to pre-treatment levels. However, in patients who were treated the longest, plasma levels of IL-6 rose to levels greater than those observed prior to initiation of treatment. Conversely, we observed a drop in plasma levels of sIL-6R and TGF- β_1 as duration of disease and treatment increased. Decreased levels of VEGF, HGF, and b-FGF were noticed after initiation of therapy. Values of sVEGF-R2 were similar in all analyzed subgroups. We evaluated whether there was a relationship between length of therapy and tested cytokines/soluble cytokine receptors in the whole group of patients with MM. We observed an inverse correlation between length of treatment and the concentration of sIL-6R, and TGF-β1 in plasma (Table 6). Furthermore, we compared the level of cytokines and soluble cytokine receptors, at various stages of disease progression. The analysis shows that the most useful parameter in evaluating the progression of multiple myeloma is the plasma level of HGF (Table 7).

Table 1. Cytokines, their soluble receptors and routine laboratory plasma levels in multiple myeloma patients and healthy controls.

Parameter	Study group				*p*-value
	Patients with MM		Healthy controls		
	n	mean±SD	n	mean±SD	
IL-6 (pg/ml)	65	13.65 ± 42.61	35	1.04 ± 1.12	0.006
sIL-6R (ng/ml)	65	37.1 ± 14.2	35	25.3 ± 6.4	0.003
VEGF (pg/ml)	65	56.8 ± 46.7	35	49.9 ± 49.9	NS
sVEGF-R2 (pg/ml)	65	7518 ± 2119	35	8725 ± 1281	<0.001
HGF (pg/ml)	76	2174 ± 2714	35	648 ± 130	<0.001
b-FGF (pg/ml)	76	7.92 ± 10.78	35	2.54 ± 5.38	<0.001
TGF- β_1 (ng/ml)	76	12.41 ± 6.64	35	13.72 ± 8.17	NS
WBC (K/µl)	66	5.56 ± 2.65	27	5.91 ± 1.38	NS
RBC (M/µl)	66	3.53 ± 0.70	27	4.80 ± 0.67	<0.001
Hb (g/dl)	66	11.2 ± 2.04	27	14.3 ± 1.94	<0.001
Hct (%)	66	32.7 ± 6.24	27	42.6 ± 5.5	<0.001
PLT (K/µl)	66	200.8 ± 86.7	27	270.1 ± 72.4	<0.001
Urea (mmol/l)	42	8.15 ± 5.98	7	5.11 ± 1.75	0.011
Creatinine (µmol/l)	42	128.5 ± 141.7	7	74.7 ± 10.99	0.020
Calcium (mmol/l)	43	2.37 ± 0.35	7	2.35 ± 0.22	NS
LDH (U/l)	37	431.4 ± 275.39	7	299.7 ± 55.79	0.012

b-FGF, b-fibroblast growth factor; Hb, haemoglobin; Hct, haematocrit; HGF, hepatocyte growth factor; IL-6, interleukin-6; LDH, lactate dehydrogenase; MM, multiple myeloma; NS, not significant; PLT, blood platelets; RBC, red blood cells; sIL-6R, soluble IL-6 receptor; SD, standard deviation; sVEGF-R2, soluble VEGF receptor; TGF-β_1, transforming growth factor-β_1; VEGF, vascular endothelial growth factor; WBC, white blood cells.

Table 2. Results of electrophoresis, CRP level, blood serum $\beta_2$microglobulin, and the plasmocyte infiltration of bone marrow in MM patients.

Parameter	Patients with MM	
	n	mean ± SD
Total Protein (g/l)	68	89.0 ± 20.8
Albumin (g/l)	68	45.8 ± 10.4
Alpha 1 globulin (%)	67	3.6 ± 1.8
Alpha 2 globulin (%)	67	10.6 ± 7.9
Beta globulin (%)	67	12.2 ± 9.2
Gamma globulin (%)	67	25.3 ± 15.3
CRP (mg/l)	17	14.95 ± 21.65
β_2 microglobulin (mg/l)	18	3.35 ± 1.76
Plasmocyte infiltration of bone marrow (%)	37	45.8 ± 27.8

CRP, C-reactive protein; MM, multiple myeloma; SD, standard deviation.

Table 3. Cytokines and their soluble receptors in male and female MM patients.

	sex	n	mean ± SD	*p*-value
IL-6 (pg/ml)	F	31	14.4 ± 43.2	0.596
	M	34	12.9 ± 42.7	
sIL-6R (ng/ml)	F	31	39.3 ± 16.8	0.975
	M	34	35.1 ± 11.2	
VEGF (pg/ml)	F	31	58.4 ± 45.7	0.519
	M	34	55.4 ± 48.1	
sVEGF-R2 (pg/ml)	F	31	7577.4 ± 2120.2	0.542
	M	34	7464.0 ± 2148.4	
HGF (pg/ml)	F	38	2021.3 ± 1783.0	0.897
	M	38	2327.8 ± 3421.8	
b-FGF (pg/ml)	F	38	5.42 ± 7.13	0.020
	M	38	10.42 ± 13.11	
TGF- β_1 (ng/ml)	F	38	11.61 ± 5.87	0.466
	M	38	13.24 ± 7.33	

b-FGF, b-fibroblast growth factor; F, female; HGF, hepatocyte growth factor; IL-6, interleukin-6; M, male; MM, multiple myeloma; sIL-6R, soluble IL-6 receptor; SD, standard deviation; sVEGF-R2, soluble VEGF receptor; TGF-β_1, transforming growth factor-β_1; VEGF, vascular endothelial growth factor;

Table 4. Comparison of levels of cytokines and soluble cytokine receptors in the treated versus untreated groups.

Parameter	Patients with MM, group N		Patients with MM, group S		p
	n	x ± SD	n	x ± SD	
IL-6 (pg/ml)	22	17.2 ± 20.7	43	11.8 ± 38.5	NS
sIL-6R (ng/ml)	22	42.9 ± 15.9	43	34.1 ± 12.4	NS
VEGF (pg/ml)	22	64.5 ± 52.6	43	52.9 ± 43.4	NS
sVEGF-R2 (pg/ml)	22	7734 ± 2007	43	7407 ± 2188	NS
HGF (pg/ml)	29	2007 ± 1660	47	2277 ± 3210	NS
b-FGF (pg/ml)	29	7.44 ±11.11	47	8.21 ± 10.69	NS
TGF- β_1 (ng/ml)	29	14.24 ± 7.00	46	11.26 ± 6.20	0.025

b-FGF, b-fibroblast growth factor; HGF, hepatocyte growth factor; IL-6, interleukin-6; MM, multiple myeloma; N, newly diagnosed patients, previously untreated; NS, not significant; S, patients remaining in treatment; sIL- 6R, soluble IL-6 receptor; SD, standard deviation; SVEGF-R2, VEGF soluble receptor; TGF-β_1, transforming growth factor-β_1; VEGF, vascular endothelial growth factor.

Table 5. Comparison of plasma levels of cytokines and soluble cytokine receptors in patient subgroups according to treatment duration.

Parameter	Group	n	mean ± SD
IL-6 (pg/ml)	N	21	18.03 ± 51.83
	S1	12	7.21 ± 7.58
	S2	9	6.34 ± 7.96
	S3	16	19.80 ± 62.30
sIL-6R (ng/ml)	N	21	43.79 ± 15.72
	S1	12	34.02 ± 12.77
	S2	9	34.02 ± 13.72
	S3	16	32.92 ± 11.29
VEGF (pg/ml)	N	21	64.87 ± 53.90
	S1	12	71.69 ± 64.67
	S2	9	39.27 ± 29.72
	S3	16	40.68 ± 23.76
sVEGF-R2 (pg/ml)	N	21	7652 ± 2019
	S1	12	7874 ± 2545
	S2	9	6985 ± 1706
	S3	16	7278 ± 2143
HGF (pg/ml)	N	28	2001 ± 1690
	S1	13	2570 ± 3489
	S2	11	2186 ± 2306
	S3	16	2073 ± 4089
b-FGF (pg/ml)	N	28	7.71 ± 11.22
	S1	13	10.54 ± 15.83
	S2	11	7.10 ± 5.97
	S3	16	5.68 ± 6.07
TGF- β_1 (ng/ml)	N	28	14.11 ± 7.09
	S1	13	11.91 ± 6.06
	S2	11	12.39 ± 7.95
	S3	16	9.60 ± 4.38

b-FGF, b-fibroblast growth factor; HGF, hepatocyte growth factor; IL-6, interleukin-6; MM, multiple myeloma; N, newly diagnosed patients, previously untreated, NS, not significant; S1, patients treated from 1 to 2 years; S2, patients treated from 2 to 3 years; S3, patients treated for longer than 3 years; sIL-6R, soluble IL-6 receptor; SD, standard deviation; sVEGF-R2, soluble VEGF receptor; TGF-β_1, transforming growth factor-β_1; VEGF, vascular endothelial growth factor.

Table 6. Analysis of correlation between duration of therapy and studied cytokines.

	n	r Spearman	p
IL-6 (pg/ml)	58	0.007	NS
sIL-6R (ng/ml)	58	-0.304	0.02
VEGF (pg/ml)	58	-0.243	NS
sVEGF-R2 (pg/ml)	58	-0.100	NS
HGF (pg/ml)	68	0.009	NS
b-FGF (pg/ml)	68	-0.083	NS
TGF- β1 (ng/ml)	68	-0.253	0.04

b-FGF, b-fibroblast growth factor; HGF, hepatocyte growth factor; IL-6, interleukin-6; NS, not significant; sIL-6R, soluble IL-6 receptor; sVEGF-R2, soluble VEGF receptor; TGF-β_1, transforming growth factor-β_1; VEGF, vascular endothelial growth factor.

Table 7. Levels of cytokines and soluble cytokine receptors in various stages of disease progression.

Parameter	Patients with MM treated with antiproliferative therapy				p
	Without disease progression		With disease progression		
	n	mean ± SD	n	mean ± SD	
IL-6 (pg/ml)	20	4.14 ± 5.75	18	19.59 ± 58.15	NS
sIL-6R (ng/ml)	20	31.2 ± 9.1	18	38.4 ± 15.4	NS
VEGF (pg/ml)	20	47.4 ± 33.9	18	55.2 ± 54.0	NS
sVEGF-R2 (pg/ml)	20	7591 ± 1612	18	7066 ± 2682	NS
HGF (pg/ml)	23	1283 ± 1583	18	3555 ± 4498	<0.001
b-FGF (pg/ml)	23	6.46 ± 9.17	18	9.71 ± 12.51	NS
TGF-β_1 (ng/ml)	22	10.84 ± 6.28	18	12.33 ± 6.33	NS

b-FGF, b-fibroblast growth factor; HGF, hepatocyte growth factor; IL-6, interleukin-6; MM, multiple myeloma; NS, not significant; sIL-6R, soluble IL- 6 receptor; SD, standard deviation; sVEGF-R2, soluble VEGF receptor; TGF-β_1, transforming growth factor-β_1; VEGF, vascular endothelial growth factor.

Discussion

In our study we showed that patients with MM demonstrate characteristically increased plasma levels of HGF, b-FGF, IL-6R and decreased levels of sVEGF-R2. Antineoplastic therapy leads to a time-dependent decrease in plasma levels of sIL-6R, and TGF-β1 in MM patients. The analysis shows that the most useful parameter in evaluating the progression of multiple myeloma is the plasma level of HGF. The data we analyzed showed that plasma concentrations of the selected cytokines are another element

which allows clinicians to evaluate disease progression as well as the effectiveness of treatment. Identifying patients, in whom treatment is less effective may be one reason to suggest a change in therapy, however this requires further study. Our study was limited by age differences in the populations studied. A correlation analysis of the studied age-dependent parameters in both groups demonstrated that age does not affect any of these parameters in patients with MM, and demonstrated that the effect is directly proportional to HGF and VEGF levels in controls (Additional file 1: supplementary Table 2). This observation led to our conclusion that HGF level played a significantly greater role than age did on the results observed.

Increased cytokine secretion by bone marrow stroma often plays a major role in the pathogenesis of some of the clinical signs of MM, such as bone disease. In our study, a statistically significant higher level of HGF, b-FGF, IL-6, and sIL-6R was observed in the blood plasma of MM patients as compared to the control group. Many studies have demonstrated that plasma levels of IL-6 and sIL-6R are elevated in patients with MM and correlate with the severity of the cancer [1,18-20]. Bataille et al. showed that in patients with stage III MM, based on the Durie and Salmon classification, levels of IL-6 were significantly higher compared to patients with stage I and II disease. In addition, it was noted that elevated levels of IL-6 in serum was a good predictor of the severity of the disease [18]. Rawstron et al. examined the expression of the CD126 antigen (α subunit of the receptor for IL-6), and found that it is only present in MM cells; it is not expressed in normal plasma cells. IL-6 leads to tumour growth, not only through direct stimulation of cell proliferation, but also via the inhibition of apoptosis of tumour plasma cells [1, 21]. Kyrstsonis et al. studied the levels of IL-6 in the serum of healthy individuals and patients with multiple myeloma treated with chemotherapy. The level of IL-6 in MM patients, which was significantly elevated before treatment started, decreased to undetectable values at the time of hematologic remission. A gradual increase in IL-6 was observed if relapse occurred [22]. In our study we observed that anti-proliferative treatment has a significant effect on sIL-6 plasma levels. The plasma level of this cytokine showed a negative correlation with the duration of treatment. Changes in the level of IL-6 in MM during treatment reflect the current ability to control the condition. The level of IL-6 decreases in the first years of treatment (group S1), when treatment is most effective. However, it increases with disease duration (S3), showing that MM progression is inevitable.

VEGF and other proangiogenic cytokines play a vital role in the neovascularization of the bone marrow of patients with MM. Vacca et al. measured the area occupied by the small blood vessels in bone marrow specimens from MM patients and compared them to healthy individuals. The area of small blood vessels was significantly larger in patients with active MM compared to patients with an inactive form of the disease, monoclonal gammopathy of undetermined significance (MGUS), or healthy subjects [23,24]. In our study plasma levels of b-FGF and VEGF in patients with MM were higher compared to the control group, but this difference was statistically significant only for b-FGF. The role of b-FGF in the pathogenesis of MM has not yet been fully described. Plowright et al. observed ectopic expression of FGFR-3 in approximately 25% of patients with MM [9]. It was observed that when cells expressing FGFR-3 were autonomous of IL-6, there was a reduced level of apoptosis and enhanced proliferative response following stimulation with IL-6. In the presence of FGF-9 and FGFR-3 ligand, test cells rapidly proliferated and were more viable than cells with normal expression of FGFR-3. Di Raimondo et al. divided patients into two groups based on the stage of their cancer (disease confined to the bone marrow vs. extramedullary location) [25]. In both groups of patients significantly elevated levels of VEGF and b-FGF were observed in the bone marrow as compared to serum. Elevated levels of VEGF and b-FGF in the bone marrow and serum of patients with MM was also reported by Zhu et al. [26]. The level of these cytokines was significantly increased in the bone marrow compared to peripheral blood, which indicates that the bone marrow is likely to be a major site of production. Many researchers have confirmed that pathological plasma cells secrete factors that stimulate angiogenesis [27-29]. In our study we observed that the mean plasma levels of sVEGF-R2 were decreased in MM patients compared to the control group. It seems that the formation of new blood vessels is probably increased in the bone marrow stroma of MM patients when compared to the control group of healthy volunteers. However, sVEGF-R2 is associated with vascular endothelial cells in healthy individuals, which seems to be confirmed by the observations obtained in the present study [27].

Studies regarding the effects of treatment on the degree of angiogenesis in multiple myeloma are inconclusive. Rajkumar et al. compared microvessel density (MVD) in 13 patients before and after treatment with high-dose chemotherapy and subsequent stem cell transplantation [30]. There was no statistically significant reduction in MVD in patients who achieved partial or complete remission after treatment. On this basis it was concluded that increased

bone marrow vascularity is not associated with regression, even in patients during complete hematologic remission. On the other hand, Sezer et al. and Sjak-Shie et al. reported a reduction of MVD in the bone marrow during chemotherapy [31,32]. Sezer et al. observed decreased MVD index in patients responding to treatment, while the index remained unchanged in the remaining patients [33]. The discrepancies in the results of the individual authors seem to indicate the limited value of MVD as an independent marker of the efficacy of anti-angiogenic therapy. During normal endothelial cell death, MVD decreases, whereas when endothelial cell death is secondary to increased neoplastic cells, MVD increases. Therefore, during anti-angiogenic therapy MVD ratio can decrease, increase or remain the same. A decrease in MVD during anti-angiogenic therapy suggests that the treatment is effective. However, lack of such an effect does not necessarily mean that the drug treatment is ineffective. The above considerations clearly indicate that using MVD as the sole parameter to determine the effectiveness of anti-angiogenic therapy is insufficient. Thus, it is important to search for another marker of angiogenesis that correlates to the clinical effect of treatment. In our study we observed significant negative correlation between bone marrow infiltration by cancer cells and the plasma level of VEGF and TGF-β1. When comparing patients in different stages of disease progression, however, variances in these markers showed no statistical significance. Thus, one can conclude that disease progression, i.e., an increase in plasmocyte infiltration of the bone marrow, correlates with a decrease in the level of VEGF and TGF-β1 in blood plasma (Additional file 1: supplementary Table 3).

Plasma levels of HGF such as those observed in our study may be useful in determining the prognosis and clinical activity in patients with MM. While examining autocrine stimulation in MM, Borset et al. reported that HGF secreted by the plasma cells stimulates the pathological c-MET receptor present on myeloma cells [14]. In addition to autocrine stimulation, Derksen et al. suggest that HGF is also produced in a paracrine manner by bone marrow stromal cells [33]. Neoplastic plasma cells present in the bone marrow, along with the "network of cytokines" and bone marrow stromal cells are jointly responsible for the destructive changes and osteolytic bone lesions. TGF-β_1 is a cytokine associated with an increase or decrease in bone osteolysis, and also has profibrotic action [33,34]. However, in our study the mean plasma levels of TGF-β_1 in MM patients and the control group did not show any statistically significant differences.

Among cytokines that were the best predictors of MM progression in our study, HGF showed very clear differences between patients grouped by clinical criteria of MM progression. HGF level was approximately two times higher in people with severe progression compared to patients without progression.

Supplementary Material

Additional File 1:
Supplementary tables 1-3.
http://www.jcancer.org/v05p0518s1.pdf

Competing Interests

The authors have declared that no competing interest exists.

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