The morphologic assessment of angiogenic growth factors and microvessel density in multiple myeloma patients.

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Background. Agniogenesis is a required step in the progression of tumor growth, invasion and metastasis. It appears to be a prominent feature of many hematological disorders, particularly in multiple myeloma (MM). Malignant plasma cells can secrete various cytokines, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) which are responsible for driving the process of neovascularization.

Material and methods. Thirty six patients with a new diagnosed MM were studied. The median age of patients at the time the samples were obtained was 60 and the range was 42-71. Clinical features of active, untreated multiple myeloma patients are shown in Table 1. Paraffin-embedded TB samples were used for immunohistochemical stains. Intensity of the positive reaction was defined according to a scale described by Kumar et al. The assessment of low-, intermediate-, and high-grade angiogenesis was based primarily on visual impression of the number of CD34 positive microvessels seen in the entire biopsy section. All patients at the time of diagnosis were divided into three groups based on International Staging System (ISS). Patients' initial treatment of MM depended on their age and comorbidities. The evaluation was done after six cycles of chemotherapy with the same methods as diagnosis or at the moment where the progression was suspected.In the control group bone marrow samples were obtained from 24 patients undergoing bone marrow biopsy for clinical indications, who were eventually classified as having normal bone marrow.

Results. In analysis of group of active, new diagnosed MM patients, we found statistically significant differences in the expression of angiogenic factors according to the group of patients after the anti-angiogenic treatment (Table 3). Additionally we found statistical differences of the expression of angiogenic factors between the group of patients with a response (PR + CR), non-active patients and the group of patients who had progression during the treatment or who did not respond to the treatment (Table 3). Concentration of MVD was significantly higher in active, new diagnosed MM patients (the median range MVD per x400 high power field was 20.16±6.76) than in the controls 2.2±0.9.The data showed statistically significant decreased in MVD after the treatment. We found a strong positive correlation between the studied factors and MVD.

Table 1. Multiple myeloma patients characteristics.

Number of patients	36
Age	60 (range 42-71)
Stage ISS I II III	 9 15 12
Solitary plasmocytoma	0
HGB [mg/dl]	10,65±1,52
Serum M Protein [g/dl]	2,26 ±1,0
Serum Albumin [g/dl]	3,9±0,53
Ca2+ [mmol/l]	2,38±0,199
IgG [mg/dl]	3471,0±1078,5
β2m [mg/l]	3879,3±1645,2
LDH [IU/l]	240,8±63,62
% plasma cells in TB	40,33±25,03

The values are presented as mean±SD, ISS- International Staging System, HGB- hemoglobin, M- monoclonal, Ca- calcium, IgG- immunoglobulin G, β2m - beta-2 microglobulin, LDH - lactate dehydrogenase, TB -trephine biopsy

Table 2. Expression of angiogenic cytokines by immunohistochemistry in active, untreated multiple myeloma patients according to the ISS.

Cytokine/cytokine receptor	No. of patients			
	Active, untreated patients, n=36	Patients before treatment		
		Patients at I ISS n=9	Patients at II ISS n=15	Patients at III ISS n=12
VEGF	49.41±16.52	46.33±14.2	48.60±18.86	52.76±19.24
bFGF	46.08±14.40	41.66±24.26	42.60±15.32	50.75±18.08
FGFR2	35.33±10.05	35.00±14.00	35.8±10.62	36.87±13.65
MVD	20.16±6.76	17.66±4.93	21.20±7.46	20.75±8.31

Values are presented as mean ±SD, ISS - International Staging System, VEGF - vascular endothelial growth factor, bFGF - basic fibroblast growth factor, FGFR2 - fibroblast growth factor receptor 2, MVD - microvessels density

Table 3. Expression of angiogenic cytokines by immunohistochemistry in active, untreated multiple myeloma patients (MM) and in MM patients after the treatment.

Cytokine/cytokine receptor		No. of patients			
			Patients after treatment		
	Active, untreated patients, n=36	After treatment, n=36	with PR+CR n=20	with stable disease n=10	with progression n=6
VEGF	49.41±16.52	29.5±9.94 #	24.62±7.26	32.32±12.81	39.25±6.99*
bFGF	46.08±14.40	35.0±11.56 ##	33.62±13.64	36.75±6.53	37.75±7.13
FGFR2	35.33±10.05	29.41±6.27	32.75±10.94	33.53±5.22	41.25±4.64**
MVD	20.16±6.76	10.50±2.27 ##	10.00±2.13	18.24±5.44	22.18±3.21***

Values are presented as mean ±SD, VEGF - vascular endothelial growth factor, bFGF - basic fibroblast growth factor, FGFR2 - fibroblast growth factor receptor 2, MVD - microvessels density (number of microvessels per x 400 field), PR- partial remission, CR- complete remission, * p=0.007 between patients with response and progression, ** p=0.05 between patients with response and progression, ***p=0.001 between patients with response and progression. #p=0.0004 between new diagnosed and after the treatment, ## p=0.02 between active, untreated patients and after treatment.

Table 4 Correlation coefficient (r) between angiogenic factors and disease features in MM patients

	VEGF	*p*	bFGF	*p*	FGFR2	*p*	MVD	*p*
HGB [mg/dl]	0.26	NS	0.14	NS	0.22	NS	0.16	0.65
Serum M Protein [g/dl]	-0.33	NS	-0.30	NS	-0.26	NS	-0.29	0.41
Serum Albumin [g/dl]	-0.009	NS	-0.15	NS	-0.14	NS	-0.07	0.82
Ca2+ [mmol/l]	0.92	0.0003	0.69	0.02	0.75	0.01	0.86	0.01
IgG [mg/dl]	-0.32	NS	-0.34	NS	-0.21	NS	-0.21	0.55
β2m [mg/dl]	-0.28	NS	-0.42	NS	-0.31	NS	-0.21	0.55
LDH [IU/l]	0.17	NS	0.12	NS	0.05	NS	0.33	0.34
(%) plasma cells in TB	0.77	0.003	0.62	0.03	0.64	0.02	0.77	0.03
MVD	0.93	<0.001	0.79	0.001	0.88	0.001	-	-

NS- not significant, HGB - hemoglobin, M - monoclonal, Ca - calcium, IgG - immunoglobulin G, β2m beta-2-microglobulin, LDH - lactate dehydrogenase, TB - trephine biopsy, MVD- microvessels density, VEGF- vascular endothelial growth factor, bFGF-basic fibroblast growth factor, FGFR2- fibroblast growth factor receptor 2.

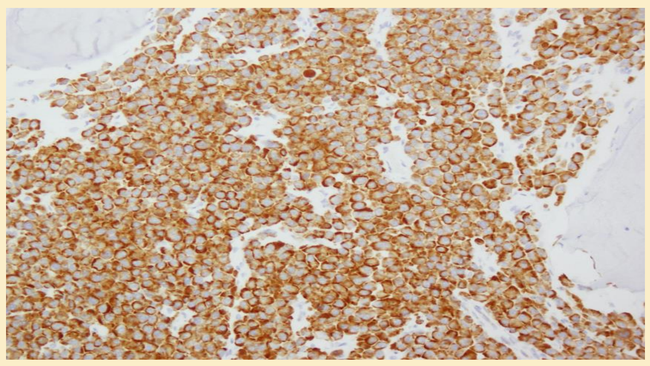

Figure 1. Immunohistochemical plasma cell clone VS38c staining of bone marrow specimen in active, untreated multiple myeloma patient with stage III according to International Staging System (x200)

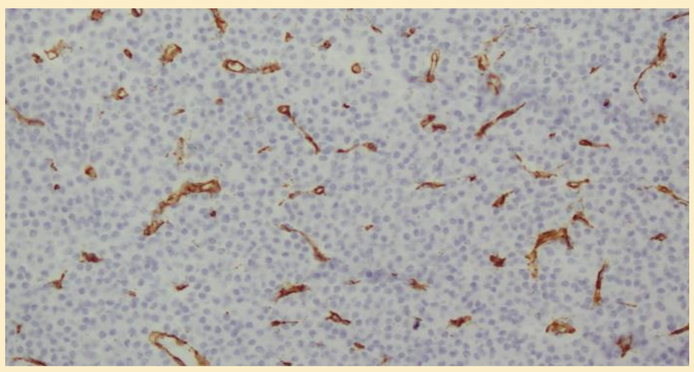

Figure 2. Immunohistochemical CD34 staining of bone marrow biopsy specimen in active, untreated MM patient with stage III according to International Staging System illustrating increased microvessels (x200).

Conclusions. The obtained results confirm increased neovascularization and increased expression of angiogenic factors in active, untreated MM patients. There are a useful markers of the response to the anti- angiogenic therapy.