Pathol. Oncol. Res. (2012) 18:681–690
DOI 10.1007/s12253-012-9495-y

RESEARCH

Angiogenesis and Survival in Patients with Myelodysplastic Syndrome

Aleksandar Savic · Vesna Cemerikic-Martinovic · Sinisa Dovat · Nebojsa Rajic · Ivana Urosevic · Borivoj Sekulic · Vanja Kvrgic · Stevan Popovic

Received: 14 April 2011 / Accepted: 3 January 2012 / Published online: 24 January 2012

Abstract Angiogenesis has been implicated in the pathogenesis and prognosis of myelodysplastic syndrome (MDS). In this study, we investigated the relationship between microvessel density (MVD), vascular endothelial growth factor (VEGF) expression, common morphological and clinical factors, and survival in patients with MDS. We examined the MVD of paraffin-embedded bone marrow sections from 70 MDS patients and 31 controls. VEGF expression was determined in 50 patients and 20 controls. The median MVD in MDS patients was significantly higher than that in controls (p=0.025), whereas there was no difference in VEGF expression between MDS patients and controls. In univariate analysis, increased MVD was associated with a shorter survival time (p=0.023). However, in multivariate analysis, MVD was not an independent predictor of survival. The VEGF expression did not influence survival in univariate analysis. Survival was independently influenced by platelet count (p=0.0073), cytogenetic risk category (p=0.022), and transfusion dependence (p=0.0073). Neither MVD nor VEGF expression were predictors for progression to acute myeloid leukemia in univariate analysis. Progression to acute myeloid leukemia was independently influenced only by the cytogenetic risk category (p=0.022). This study confirmed increased MVD in MDS. It does not support an independent prognostic role of angiogenesis in MDS.

Keywords Myelodysplastic syndrome · Angiogenesis · Microvessel density · VEGF · Survival · Prognosis

Abbreviations

ALIP	Atypical localization of immature progenitor cells
AML	Acute myeloid leukemia
AML-MRC	Acute myeloid leukemia with myelodysplasia-related changes
CMML	chronic myelomonocytic leukemia
FAB	French-American-British
H&E	hematoxylin and eosin
IPSS	International Prognostic Scoring System
MDS	Myelodysplastic syndrome
MVD	Microvessel density
RA	Refractory anemia
RAEB	Refractory anemia with excess blasts
RAEB-T	Refractory anemia with excess blasts in transformation
RARS	Refractory anemia with ringed sideroblasts
RCMD	Refractory cytopenia with multilineage dysplasia
RCUD	Refractory cytopenia with unilineage dysplasia
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
WPSS	WHO classification-based prognostic scoring system

A. Savic (✉) · N. Rajic · I. Urosevic · B. Sekulic · V. Kvrgic · S. Popovic
Clinic of Hematology, Clinical Center of Vojvodina,
Faculty of Medicine,
Hajduk Veljkova 1-3,
21000 Novi Sad, Serbia
e-mail: asavic@uns.ac.rs

V. Cemerikic-Martinovic
Pathohistology Laboratory Beolab,
Resavska 60,
11000 Belgrade, Serbia

S. Dovat
College of Medicine, Milton S. Hershey Medical Center,
Children's Hospital, Department of Pediatrics, H085, Division of Pediatric Hematology/Oncology, Pennsylvania State University,
500 University Drive, P.O. Box 850, Hershey, PA 17033, USA

Introduction

Myelodysplastic syndrome (MDS) represents a heterogeneous group of acquired clonal hematopoietic disorders that lead to bone marrow failure and an increased risk of transformation to acute myeloid leukemia (AML). Angiogenesis is a formation of new blood vessels from pre-existing vessels [1]. It has a major role in tumor growth and spread [1, 2]. Several studies have suggested that increased microvessel density (MVD) plays a role in hematological disorders, including acute leukemia [3, 4], myeloproliferative diseases [5, 6], chronic lymphocytic leukemia [7], multiple myeloma [8], and MDS [9–13]. Vascular endothelial growth factor (VEGF) plays a crucial role in angiogenesis by binding to the tyrosine kinase receptors VEGFR-1 and VEGFR-2 [14]. VEGF has been shown to be expressed in bone marrow blast cells, and an autocrine mechanism of VEGF signaling has been established in MDS [15, 16]. In this study, we investigated the relationship between microvessel density, VEGF expression, common morphological and clinical factors, and survival in patients with MDS.

Materials and Methods

Patients

Paraffin-embedded bone marrow biopsies obtained from 70 MDS patients at the time of diagnosis between 1990 and 2009 were studied. Control bone marrow samples from 31 subjects with no evidence of marrow disease also were evaluated; these biopsies were performed as part of the staging procedure for Hodgkin's disease (15 cases), non-Hodgkin's lymphomas (13 cases), and other diseases (hereditary hemochromatosis, exudative pericarditis and lumbar syndrome). The study was approved by the institutional ethics committee and informed consent was obtained from patients. The classification criteria were established according to French-American-British (FAB) classification and World Health Organization (WHO) classification and confirmed independently by two hematologists [17, 18]. The number of blast cells was determined on May-Grunwald-Giemsa stained bone marrow smears and was expressed as percentage of 500 counted cells [17, 18]. Patients with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) were considered to have low-risk MDS, whereas patients with chronic myelomonocytic leukemia (CMML), refractory anemia with excess blasts (RAEB), and refractory anemia with excess blasts in transformation (RAEB-T) were classified as having high-risk disease. The International Prognostic Scoring System (IPSS) was applied for 42 out of 70 patients for whom cytogenetic data were available [19]. Data on clinical outcome (death, survival, and development of AML) and other clinical and laboratory characteristics were collected from patients' medical files.

Bone Marrow Histology and Immunohistochemical Examination

Bone marrow pathological specimens were fixed in buffered formalin (or B5 fixative for some controls), decalcified with HCl, dehydrated in ethanol and chloroform, and embedded in paraffin. Serial sections (4–6 μm thick) of each sample were processed for hematoxylin and eosin (H&E) and reticulin staining and for immunohistochemical identification.

Before staining, tissue sections were deparaffinized in xylol and rehydrated in a graded ethanol series. Antigen retrieval was performed using citrate solution in a microwave oven, and inhibition of endogenous peroxidase was performed with H_2O_2 treatment. Immunostaining was conducted using the labeled streptavidin-biotin peroxidase method (DAKO LSAB, Dako, Carpinteria, CA, USA) with 3-amino-9-ethylcarbazole (AEC) as the substrate chromogen. Bone marrow samples were stained for CD34 with the monoclonal antibody Dako QBEnd-10 at a final of concentration of 1:50. An antibody for VEGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used at a final concentration of 1:50. Finally, counterstaining with hematoxylin was performed. The VEGF and CD34 expression levels were expressed as percentages of 500 counted cells. The VEGF expression was determined in 50 MDS patients (out of 70, treated between 1990 and 2002) and in 20 controls (out of 31, Hodgkin's disease 9 cases, non-Hodgkin's lymphomas 9 cases, and other diseases 2 cases). The CD34 expression was determined in all patients and controls.

The cellularity and fibrosis in the bone marrow biopsy samples were determined following the European consensus guidelines [20].The atypical localization of immature progenitor cells (ALIP) were determined according to Tricot et al. [21].

Microvessel Staining and Counting

Microvessel density (MVD) was quantified as the number of blood vessels per high power field counted using light microscopy at 400× magnification. Well-vascularized areas were chosen for quantification, and for each patient multiple areas were counted covering almost the whole of the specimen and averaged [22]. For MDS patients, the number of counted areas ranged from 3 to 17 (median, 7 per patient). The median number of counted areas in the control group was 9 and ranged from 5 to 17. Blood vessels were identified as CD34-positive cells that were morphologically compatible with endothelial cells and separate from other

microvessels forming a cluster, even in the absence of a recognizable lumen. Arterioles were excluded on the basis of the presence of media.

Statistical Analysis

The significance of differences between the groups of patients was determined using the Mann–Whitney *U* test and Kruskal-Wallis ANOVA by Ranks (Kruskal-Wallis ANOVA). We used the Spearman rank correlation test to assess the relationships among parameters. Survival was plotted using Kaplan–Meier plots. Survival curves were compared using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards regression to identify significant independent prognostic factors. A p-value of < 0.05 was considered to be statistically significant.

Results

Clinical and Laboratory Characteristics of MDS Patients

Seventy MDS patients were enrolled in this study, including 51 males and 19 females with a median age of 66 years (range, 33 to 80). According to the FAB classification, 18 patients were classified as RA, four patients as RARS, 34 patients as RAEB, three patients as RAEB-T and 11 patients as CMML. Based on WHO criteria, two patients were classified as refractory cytopenia with unilineage dysplasia (RCUD/RA), three patients as RARS, 17 patients as refractory cytopenia with multi-lineage dysplasia (RCMD) including a patient with ringed sideroblasts (RCMD-RS), 16 patients as RAEB-1, 18 patients as RAEB-2, 11 patients as CMML-1, and three patients as AML with myelodysplasia-related changes (AML-MRC). Table 1 shows the data for peripheral blood counts, the percentage of bone marrow blasts, and bone marrow cellularity according to the FAB and WHO classification. According to the IPSS, cytogenetic abnormalities were categorized into three groups: good prognosis (31 patients, 74%), intermediate prognosis (5 patients, 12%), and poor prognosis (6 patients, 14%). Of the 42 patients, 7 were IPSS low risk (16.66%), 20 were IPSS Int-1 (47.62%), 10 were Int-2 (23.81%), and 5 were high risk (11.90%). The transformation to AML occurred in 22 patients (33%).

Microvessel Density

Figure 1 shows the MVD in MDS patients and controls. The median MVD in MDS patients was 3.83 (range, 0.00 to 49.40), which was significantly higher than that in controls (median, 2.00; range, 0.00 to 5.61) (p=0.025, Mann–Whitney *U* test) (Fig. 2a and b). The values for MVD according to FAB and WHO classification are given in Table 2. The MVD values were higher in MDS patients from the high-risk group (median, 4.05; range, 0.00 to 49.44) compared to those from the low-risk group (median, 3.065; range, 0.14 to 13.71). However, the difference between the two groups was not significant.

When we analyzed the relationship between MVD and the above-mentioned clinical, laboratory, and morphological parameters, Spearman rank correlation analysis revealed significant correlation between MVD and hemoglobin concentration (p=0.0397) and MVD and CD34 expression (p= 0.000198). The Kruskal-Wallis ANOVA did not find any differences when we looked at MVD in different IPSS, FAB and WHO groups.

VEGF and CD34 Expression

The median VEGF expression in bone marrow biopsies from MDS patients was 8.30% (range, 4.00% to 50.20%), and the median VEGF expression in controls was 12.15% (range, 3.00% to 37.00%). The difference between MDS patients and controls was not significant (Mann–Whitney *U* test). The VEGF expression was higher in the low-risk MDS group (median, 10.04%; range, 0.40% to 50.20%) compared with the high-risk group (median, 7.4%; range, 0.40% to 34.40%), but the difference was not significant (Mann–Whitney *U* test). The values for VEGF expression according to FAB and WHO classification are given in Table 3.

VEGF expression was detected in cytoplasm of blast cells, promyelocytes, myelocytes, monocytes, some erythroblasts, lymphoid and plasma cells in MDS patients as well as in controls. VEGF expression was rarely detected in megakaryocytes and mature granulocytes in MDS patients but none was found in controls (Fig. 2c and d).

We did not find significant correlation between VEGF expression and any other clinical, laboratory, or morphological parameter. There were no significant differences in VEGF expression between IPSS, FAB and WHO groups (Kruskal-Wallis ANOVA).

The median CD34 expression in bone marrow biopsies from MDS patients was 1.1% (range, 0.00% to 16.40%), which was higher than that in controls (median, 0.8%; range, 0.0% to 1.60%). The difference between MDS patients and controls was significant (p=0.0085, Mann–Whitney *U* test). Table 2 provides the values for CD34 expression according to FAB and WHO classification. CD34 expression was higher in MDS patients from the high-risk group (median, 1.6%; range, 0.00% to 16.4%) compared to the low-risk group (median, 0.65%; range, 0% to 5.2%), but the difference was not significant (Mann–Whitney *U* test). CD34 expression was correlated with medullar blast count (p=0.012), platelet number (p=

Table 1 Hematological and pathohistological values of the MDS patients according to FAB and WHO group

	Patients (no.)	Haemoglobin[a] (g/l)	White blood cells [a] ($x10^9$/l)	Platelets[a] ($x10^9$/l)	Bone marrow blast cells[a] (%)	Bone marrow cellularity [a] (%)
FAB group						
RA	18	85.17±20.61	3.75±1.47	202.17±178.15	2.07±1.46	56.47±23.17
RARS	4	80.95±27.99	4.05±1.64	287.00±136.05	1.83±1.65	51.33±16.84
RAEB	34	82.11±21.53	3.52±2.39	118.96±98.50	10.55±4.48	64.72±19.89
RAEB-T	3	90.95±26.94	2.55±0.86	80.65±63.00	23.43±1.29	58.42±29.35
CMML	11	93.13±29.38	13.03±4.64	100.79±45.32	4.33±3.30	75.09±17.86
WHO group						
RCUD/RA	2	91.55±7.85	4.25±2.33	239.3±67.46	2.25±2.47	65±42.43
RARS	3	93.00±17.44	4.73±1.10	319.33±146.60	2..10±1.91	50.11±20.41
RCMD	17	82.05±23.10	3.58±1.44	197.09±182.34	1.98±1.39	55.31±21.01
RAEB-1	16	86.85±21.23	3.95±2.49	151.64±99.70	7.00±1.86	65.30±16.45
RAEB-2	18	77.90±21.51	3.14±2.29	89.90±90.36	14.65±2.66	64.20±22.99
CMML-1	11	93.13±29.38	13.03±4.64	100.79±45.32	4.33±3.30	75.09±17.86
AML-MRC	3	90.95±26.94	2.55±0.86	80.65±63.00	23.43±1.29	58.42±29.35
All patients	70	84.73±22.67	5.00±4.24	147.07±129.74	7.37±6.33	63.29±21.16

[a] Mean and S.D. values

0.0032), and MVD (p=0.0002). The Kruskal-Wallis ANOVA revealed significant differences in CD34 expression between FAB groups (p=0.0057) as well as between WHO groups (p=0.0225).

Survival and Prognosis

The median follow-up time was 21 months (range, 1.3 to 154 months), and the median survival was 24 months. Fifty-two patients died (78%) and three patients were lost from the follow-up. For the survival analysis, we analyzed continuous variables by dividing the patients on the basis of median values, whereas category variables were grouped (Table 4). In univariate survival analysis using the log-rank method, patients with MVD<3.83 (below the median value) had a significantly better survival rate than those with MVD≥3.83 (p=0.023). Figure 3 shows the overall survival in relation to MVD. The other adverse prognostic factors for

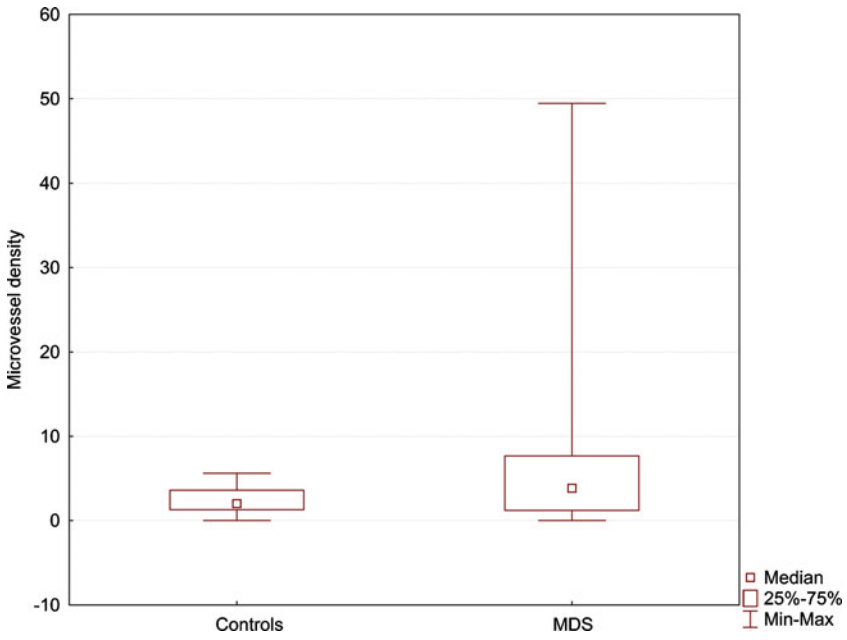

Fig. 1 MVD in MDS and control patients

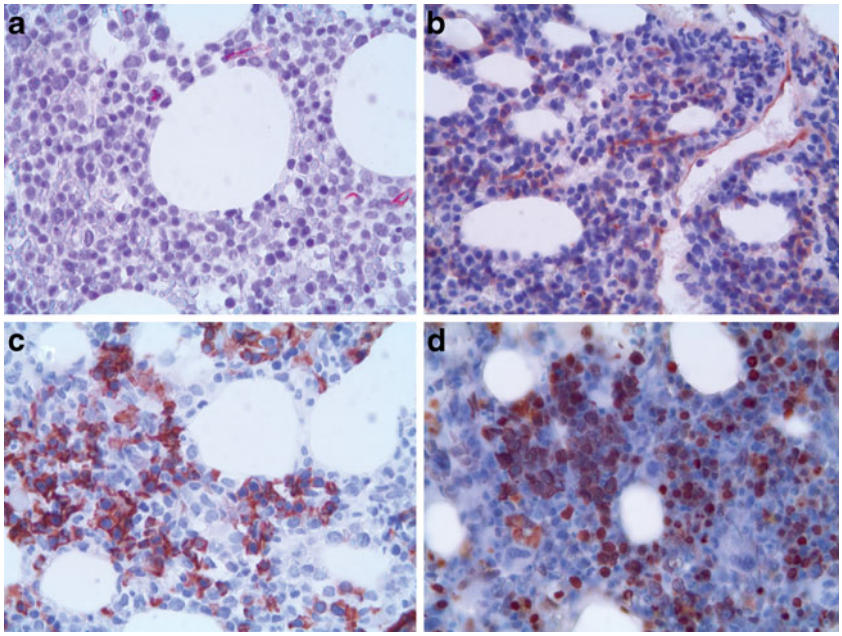

Fig. 2 Immunohistochemical staining of bone marrow sections of MDS and control group. a MVD (CD34 staining) in the control group. b MVD (CD34 staining) in a patient with MDS (RAEB) (original magnification 400 x). c VEGF staining in the control group. d VEGF staining in a patient with MDS (RAEB) (original magnification 400 x)

overall survival in univariate analysis were: transfusion dependence, elevated medullary blast count (> 5.7%), IPSS poor cytogenetic group, FAB high-risk group, platelet number (< 113.9×10^9/L), hypercellular bone marrow, and presence of ALIP. VEGF expression (Fig. 4) and CD34 expression did not show prognostic significance for overall survival (Table 4).

All parameters that exhibited prognostic significance by univariate testing were included in the multivariate analysis. In multivariate analysis using the Cox proportional hazard model, survival was independently influenced by platelet count (p=0.0073), cytogenetic risk category (p=0.022), and transfusion dependence (p= 0.0073). MVD did not have independent prognostic significance in multivariate analysis. We also assessed the impact of all of the parameters analyzed at diagnosis on the progression of MDS to AML (Table 4). In univariate analysis, MVD did not influence progression to AML, whereas transfusion dependency, elevated medullary blast count (> 5.7%), IPSS poor cytogenetic group, FAB high-risk group, female gender, and the presence of ALIP had adverse prognostic significance. In multivariate analysis, progression to AML was independently influenced only by the cytogenetic risk category (p=0.022).

Discussion

The increased MVD in MDS patients compared with a control group was determined in this study as well as in previous studies [9–13, 23]. Several studies showed significantly increased MVD in high-risk MDS groups compared to low-risk MDS groups [10, 13]. Although we found increased MVD in high-risk MDS patients compared with low-risk patients, the difference was not statistically significant, possibly because of the small number of patients in some of the FAB groups. This was also the case in other studies [11, 12].

In our study, the level of VEGF expression did not differ significantly between the MDS and control groups or between FAB and WHO groups, which is in contrast to a previous study that reported higher expression in high-risk MDS [16]. Such conflicting results could be the consequence of different methods used. The common finding in our study and previous studies [15, 16] is that VEGF expression occurs in immature granulocytic cells, monocytes and blast cells in MDS.

To the best of our knowledge, this is the second study reporting a significant negative correlation between MVD and overall survival in univariate analysis. The prognostic impact of MVD in univariate analysis on the survival of MDS patients was reported previously by Alexandrakis et al. [13] but not in other studies [11, 12]. Korkolopoulou et al. [11] found that survival is correlated with size-related morphometric parameters of angiogenesis. In multivariate analysis, MVD does not seem to be an independent adverse prognostic factor [this study, 13]. The independent prognostic factors for survival in our study were karyotype, transfusion dependence and platelet count. These factors take part in highly accepted prognostic models in MDS, IPSS and WHO classification-based prognostic scoring system (WPSS) [19, 24]. VEGF expression did not have prognostic significance for survival. However, high intracellular VEGF concentration determined by Western

Table 2 MVD and CD34 expression in MDS patients according to FAB and WHO group

	Patients (no.)	Microvessel density[a] (MVD)	CD34 positive cells/500 cells[a] (%)
FAB group			
RA	18	3.065	0.50
		0.14–13.71	0.00–5.20
RARS	4	5.70	0.75
		0.33–12.70	0.20–2.00
RAEB	34	3.52	1.50
		0.00–49.44	0.20–16.40
RAEB-T	3	5.40	3.80
		3.33–8.27	0.80–5.60
CMML	11	4.40	1.80
		0.71–15.20	0.00–5.20
WHO group			
RCUD/RA	2	0.87	0.60
		0.75–1.00	0.20–1.00
RARS	3	10.64	0.80
		0.33–12.70	0.20–2.00
RCMD	17	3.33	0.60
		0.14–13.71	0.00–5.20
RAEB-1	16	4.00	1.30
		0.00–49.44	0.20–8.80
RAEB-2	18	3.52	1.70
		0.40–10.20	0.20–16.40
CMML-1	11	4.40	1.80
		0.71–15.20	0.00–5.20
AML-MRC	3	5.40	3.80
		3.33–8.27	0.80–5.60
All patients	70	3.83	1.10
		0–49.44	0–16.40

[a] Median and range values

Table 3 VEGF expression in MDS patients according to FAB and WHO group

	Patients (no.)	VEGF positive cells/500 cells[a] (%)
FAB group		
RA	16	10.45
		0.40–50.20
RARS	2	14.80
		5.00–24.60
RAEB	20	7.40
		0.80–27.40
RAEB-T	2	15.80
		6.80–24.80
CMML	10	7.60
		0.40–34.40
WHO group		
RCUD/RA	2	0.6
		0.40–0.80
RARS	1	24.60
		–
RCMD	15	11.60
		4.30–50.02
RAEB-1	10	3.65
		0.80–27.40
RAEB-2	10	9.80
		1.60–23.20
CMML-1	10	7.60
		0.40–34.40
AML-MRC	2	15.80
		6.80–24.80
All patients	50	8.3
		0.4–50.2

[a] Median and range values

blotting and the radioimmunoassay (RIA) method was reported to be an adverse prognostic factor for survival in MDS [25].

Increased MVD and VEGF expression could promote clonal hematopoiesis by providing an adequate microvascular network, particularly in patients with low-risk MDS. On the other hand, VEGF can stimulate leukemic growth by paracrine and autocrine mechanisms in high-risk MDS patients [15, 16, 25–28].

In our study, MVD was not a prognostic factor for progression to AML, which disagrees with the results of Korkolopoulou et al. [11]. VEGF did not affect AML progression either; to our knowledge that was not previously investigated. Concerning progression to AML karyotype was an independent prognostic factor which is in line with previous results [19, 24, 29, 30].

Several researchers have suggested that MVD could be considered in diagnostic histology and immunohistochemistry in MDS [31, 32]. Whether angiogenesis in MDS has practical significance outside of the prognostic field remains to be seen. Although angiogenesis was increased in MDS patients compared to controls in our study, about two-thirds of the MDS patients had MVD values below the maximum value in the control group. MVD is also increased in other non-malignant conditions that in many ways resemble MDS, such as HIV infection [33]. Data about MVD are lacking for other non-malignant conditions that are infective, autoimmune, or toxic in nature and that could make differential diagnosis for MDS problematic. Thus, determination of MVD in routine histology likely would not make a substantial difference in differential diagnosis of MDS.

Table 4 Prognostic factors for overall survival/AML progression (log-rank analysis)

Prognostic factors[a]	Median overall survival	Overall survival p-value	Probability of AML progression (%)		AML progression p-value
			2 years	5 years	
Microvessel density		0.023			0.44
≥3.83	21		41	47	
<3.83	36		31	43	
VEGF expression%		0.21			0.09
≥8.3	18		30	40	
<8.3	21		25	65	
CD34 expression%		0.14			0.54
≥1.1	24		40	40	
<1.1	27		32	40	
Bone marrow blasts%		0.024			0.001
≥5.7	17		58	66	
<5.7	36		16	24	
Age (years)		0.22			0.45
≥66	22		32	46	
<66	34		39	50	
Sex		0.80			0.022
Male	26		30	39	
Female	24		54	83	
Hemoglobin concentration (g/L)		0.44			0.22
≥86.8	27		46	56	
<86.8	24		23	34	
Platelet count (x 10^9/L)		0.0153			0.20
≥113.9	34		28	35	
<113.9	19		49	49	
Polymorphonuclear cell count (x 10^9/L)		0.52			0.38
≥1.39	25		47	56	
<1.39	19		31	31	
Bone marrow cellularity		0.0082			0.14
Normal	79		27	27	
Hypercellularity	19		41	63	
Hypocellularity	48		25	25	
Karyotype (IPSS)		0.016			0.043
Good	42		17	31	
Intermediate	13		75	75	
Unfavourable	6		75	75	
Transfusion dependence		0.00003			0.0032
Yes	21		42	42	
No	Not reached		0	0	
FAB risk group		0.003			0.001
Low risk (RA+RARS)	47		11	22	
High risk (RAEB, RAEB-T, CMML)	17		50	55	
Bone marrow fibrosis		0.41			0.80
Grade 1+2	25		36	41	
Grade 3+4	26		24	39	
ALIP		0.016			0.007
Yes	17		56	60	
No	34		16	16	

[a] Continuous variables were analysed by dividing the patients on the basis of median values

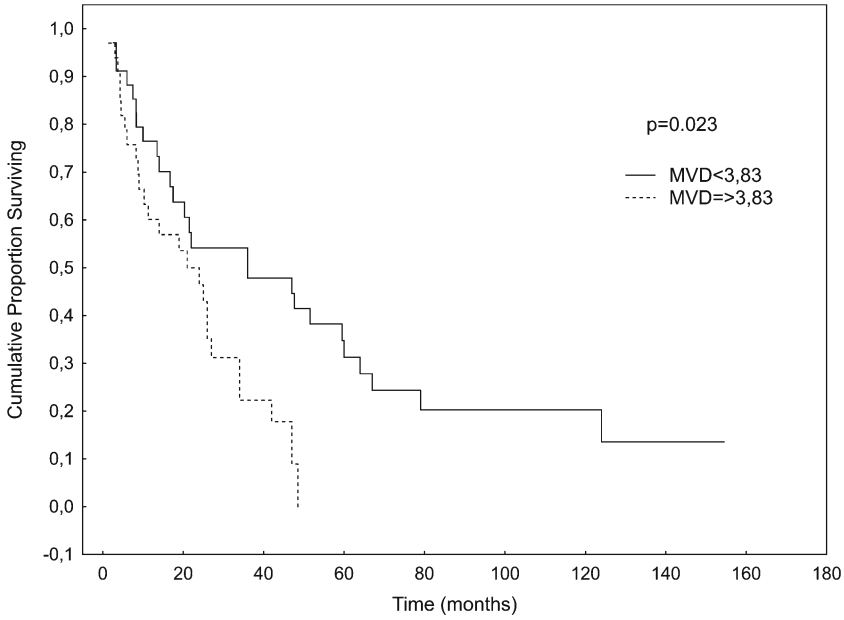

Fig. 3 Survival in MDS patients according to MVD. Patients with MVD <3.83 (solid line) and ≥3.83 (dashed line). The patients are divided in groups according to the observed median value. The x-axis shows survival in months

Angiogenesis has been a potential treatment target for MDS. Lenalidomide and thalidomide are immunomodulatory agents that have shown clinical activity in MDS; they have multiple biological effects on hematopoiesis, including anti-angiogenic effects [34–36]. However, anti-angiogenic agents, including SU5416 and AG-013736 (small molecules that inhibit the VEGF-receptor tyrosine kinases) and bevacizumab (a recombinant, anti-VEGF, humanized, monoclonal antibody), had limited clinical activity in MDS [37–39]. Thus, whether angiogenesis is a feasible target for treating MDS requires further research.

In conclusion, this study confirmed increased MVD as part of the pathogenesis of MDS. In univariate survival analysis, increased MVD was an adverse prognostic factor for survival, but in multivariate analysis it did not have an independent prognostic significance. VEGF was not a prognostic factor for overall survival. Neither MVD nor VEGF expression were predictors for progression to acute myeloid leukemia in univariate analysis. The common prognostic variables, such as cytogenetics, transfusion dependence and platelet count had an independent prognostic value for survival and seem to be well associated with basic mechanisms of MDS.

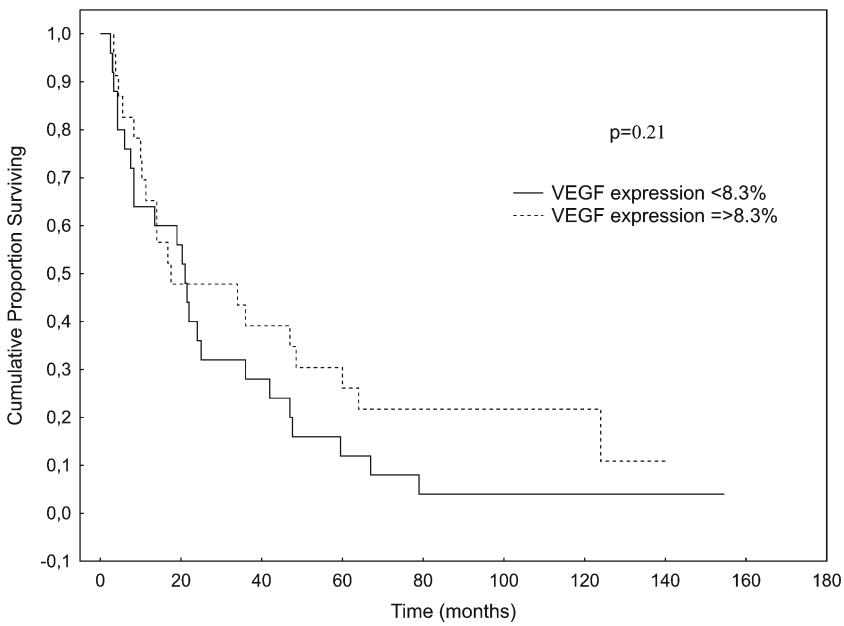

Fig. 4 Survival in MDS patients according to VEGF expression. Patients with VEGF expression<8.3% (solid line) and≥8.3% (dashed line). The patients are divided in groups according to the observed median value. The x-axis shows survival in months

Acknowledgments We wish to thank Dusan Pejin and Nada Vuckovic for their support.

Contributors: All authors have actively participated to the study and approved the manuscript. Aleksandar Savic designed the research, collected patient information, analyzed and interpreted the data, and wrote the manuscript. Vesna Cemerikic-Martinovic contributed control patients, reviewed bone marrow histology. Sinisa Dovat provided critical revisions. Borivoje Sekulic and Vanja Kvrgic collected patient information. Ivana Urosevic and Nebojsa Rajic collected patient information, and contributed patients. Stevan Popovic contributed patients.

Conflicts of interest statement There are no conflicts of interests to disclose.

References

1. Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid, and other disease. Nat Med 1:27–31
2. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–1186
3. Padro T, Ruiz S, Bieker R et al (2000) Increased angiogenesis in the bone marrow of patients with acute myeloid leukemia. Blood 95:2637–2644
4. Hussong JW, Rodgers JM, Shami PJ (2000) Evidence of increased angiogenesis in patients with acute myeloid leukemia. Blood 95:309–313
5. Lundberg LG, Lerner R, Sundelin P et al (2000) Bone marrow in polycythemia vera, chronic myelocytic leukemia, and myelofibrosis has an increased vascularity. Am J Pathol 157:15–19
6. Mesa RA, Hanson CA, Rajkumar SV et al (2000) Evaluation and clinical correlations of bone marrow angiogenesis in myelofibrosis with myeloid metaplasia. Blood 96:3374–3380
7. Peterson L, Kini AR (2001) Angiogenesis is increased in B-cell chronic lymphocytic leukemia. Blood 97:2529
8. Rajkumar SV, Leong T, Roche PC et al (2000) Prognostic value of bone marrow angiogenesis in multiple myeloma. Clin Cancer Res 6:3111–3116
9. Aguayo A, Kantarjian H, Manshouri T et al (2000) Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood 96:2240–2245
10. Pruneri G, Bertolini F, Soligo D et al (1999) Angiogenesis in myelodysplastic syndromes. Br J Cancer 81:1398–1401
11. Korkolopoulou P, Apostolidou E, Pavlopoulos PM et al (2001) Prognostic evaluation of the microvascular network in myelodysplastic syndromes. Leukemia 15:1369–1376
12. Lundberg LG, Hellstrom-Lindberg E, Kanter-Lewensohn L et al (2006) Angiogenesis in relation to clinical stage, apoptosis and prognostic score in myelodysplastic syndromes. Leuk Res 30:247–253
13. Alexandrakis MG, Passam FH, Pappa CA et al (2005) Relation between bone marrow angiogenesis and serum levels of angiogenin in patients with myelodysplastic syndromes. Leuk Res 29:41–46
14. Gale NW, Yancopoulos GD (1999) Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. Genes Dev 13:1055–1066
15. Bellamy WT, Richter L, Sirjani D et al (2001) Vascular endothelial cell growth factor is an autocrine promoter of abnormal localized immature myeloid precursors and leukemia progenitor formation in myelodysplastic syndromes. Blood 97:1427–1434
16. Wimazal F, Krauth MT, Vales A et al (2006) Immunohistochemical detection of vascular endothelial growth factor (VEGF) in the bone marrow in patients with myelodysplastic syndromes: correlation between VEGF expression and the FAB category. Leuk Lymphoma 47:451–460
17. Bennett JM, Catovsky D, Daniel MT et al (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51:189–199
18. Brunning RD, Orazi A, Germing U et al (2008) Myelodysplastic syndromes/Neoplasms, overview. In: Swerdlow SH, Campo E, Harris NL et al (eds) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC, Lyon, pp 88–93
19. Greenberg P, Cox C, LeBeau MM et al (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088
20. Thiele J, Kvasnicka HM, Facchetti F et al (2005) European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 90:1128–1132
21. Tricot G, De Wolf-Peeters C, Vlietinck R et al (1984) Bone marrow histology in myelodysplastic syndromes. II. Prognostic value of abnormal localization of immature precursors in MDS. Br J Haematol 58:217–225
22. Perez-Atayde AR, Sallan SE, Tedrow U et al (1997) Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. Am J Pathol 150:815–821
23. Keith T, Araki Y, Ohyagi M et al (2007) Regulation of angiogenesis in the bone marrow of myelodysplastic syndromes transforming to overt leukaemia. Br J Haematol 137:206–215
24. Malcovati L, Germing U, Kuendgen A et al (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25:3503–3510
25. Verstovsek S, Estey E, Manshouri T et al (2002) Clinical relevance of vascular endothelial growth factor receptors 1 and 2 in acute myeloid leukaemia and myelodysplastic syndrome. Br J Haematol 118:151–156
26. Gabrilove J (2001) Angiogenic growth factors: autocrine and paracrine regulation of survival in hematologic malignancies. Oncologist 6(Suppl 5):4–7
27. Dias S, Hattori K, Zhu Z et al (2000) Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. J Clin Invest 106:511–521
28. Padro T, Bieker R, Ruiz S et al (2002) Overexpression of vascular endothelial growth factor (VEGF) and its cellular receptor KDR (VEGFR-2) in the bone marrow of patients with acute myeloid leukemia. Leukemia 16:1302–1310
29. Germing U, Hildebrandt B, Pfeilstöcker M et al (2005) Refinement of the international prognostic scoring system (IPSS) by including LDH as an additional prognostic variable to improve risk assessment in patients with primary myelodysplastic syndromes (MDS). Leukemia 19:2223–2231
30. Sperr WR, Wimazal F, Kundi M et al (2001) Survival analysis and AML development in patients with de novo myelodysplastic syndromes: comparison of six different prognostic scoring systems. Ann Hematol 80:272–277
31. Horny HP, Sotlar K, Valent P (2007) Diagnostic value of histology and immunohistochemistry in myelodysplastic syndromes. Leuk Res 31:1609–1616
32. Valent P, Horny HP, Bennett JM et al (2007) Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: consensus statements and report from a working conference. Leuk Res 31:727–736
33. Patsouris E, Katsarou O, Korkolopoulou P et al (2004) Increased microvascular network in bone marrow of HIV-positive haemophilic patients. HIV Med 5:18–25
34. List A, Dewald G, Bennett J et al (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355:1456–1465

35. Raza A, Reeves JA, Feldman EJ et al (2008) Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. Blood 111:86–93
36. Musto P (2004) Thalidomide therapy for myelodysplastic syndromes: current status and future perspectives. Leuk Res 28:325–332
37. Giles FJ, Stopeck AT, Silverman LR et al (2003) SU5416, a small molecule tyrosine kinase receptor inhibitor, has biologic activity in patients with refractory acute myeloid leukemia or myelodysplastic syndromes. Blood 102:795–801
38. Giles FJ, Bellamy WT, Estrov Z et al (2006) The anti-angiogenesis agent, AG-013736, has minimal activity in elderly patients with poor prognosis acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). Leuk Res 30:801–811
39. Oh ST, Gotlib J (2008) Antiangiogenic therapy in myelodysplastic syndromes: is there a role? Curr Hematol Malig Rep 3:10–18