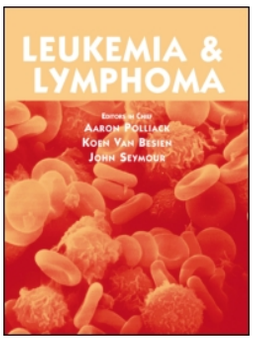

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Vascular endothelial growth factor overexpression in myelodysplastic syndrome bone marrow cells: biological and clinical implications

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ABSTRACT

In myelodysplastic syndrome (MDS), vascular endothelial growth factor (VEGF) may have regulatory effects on the hematopoietic system and contribute to disease progression. We analyzed by immunocytochemistry VEGF expression in bone marrow (BM) cells from 188 patients with MDS and 96 non-hemopathic subjects. We also measured VEGF BM plasma levels and *in vitro* VEGF release. Our aims were to evaluate whether VEGF expression abnormalities were associated with relevant laboratory or clinical findings and their possible prognostic value. In MDS, VEGF expression was higher than in controls ($p < .0001$) and VEGF release was significantly higher in the low-risk cases. A trend to a positive correlation between VEGF myeloid expression and apoptotic rate was observed. High myeloid VEGF levels were independently associated with longer overall survival ($p < .0001$) and progression-free survival ($p = .0002$). Our findings suggest that, in MDS, VEGF production and release may contribute to ineffective hematopoiesis, with a potential prognostic role.

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KEYWORDS
Vascular endothelial growth factor; bone marrow; myelodysplastic syndrome; prognosis

Introduction

Angiogenic factors influence the growth and differentiation of hematopoietic cells in normal conditions as well as in hematologic malignancies. Most angiogenic factors appear to be secreted by hematopoietic cells, with effects of autocrine or paracrine regulation on the hematopoietic system.

An abnormal angiogenesis seems involved in the pathogenesis of leukemia and myelodysplasia as well as of solid tumors.[1] In acute myeloid leukemia (AML), it was observed an increase in bone marrow (BM) angiogenesis at diagnosis and its normalization at the remission of the disease. In various neoplasias, myelodysplastic syndrome (MDS) included, angiogenesis is associated with the presence of circulating endothelial cells (CECs), and the expression of various angiogenesis mediators has been found to be altered.[2,3]

Vascular endothelial growth factor (VEGF) is a 34- to 42-kDa dimeric multifunctional glycoprotein with various isoforms whose gene is located on chromosome 6p21.3.[4,5] The smaller isoforms, VEGF165 and VEGF121, are secreted proteins and act as diffusible agents, whereas the larger isoforms, VEGF189 and VEGF206, remain cell associated.[6,7] VEGF is the most important growth factor of endothelial cells and exerts its effect via tyrosine kinase receptors.[8]

VEGF expression correlated with the increased vessel density found in AML.[9,10] It also plays a major role in MDS. Intense coexpression of VEGF and its receptors was detected in myeloblasts and immature myeloid BM cells of MDS and it was hypothesized that autocrine production of this angiogenic peptide might contribute to inflammatory cytokine elaboration, leukemia progenitor self-renewal and disease progression in the MDS subtypes at high risk of leukemic evolution.[11–14]

However, the studies concerning clinical implication of VEGF expression in MDS are limited and the prognostic significance of this angiogenic factor remains controversial. Whereas some authors found that VEGF expression was predictor for progression to AML, other studies did not support an independent prognostic role of VEGF overexpression in MDS.[15]

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We analyzed by immunocytochemistry VEGF expression in BM cells from a group of patients with MDS, not previously treated, and a group of non-hemopathic subjects. We also measured by an immunoassay VEGF BM plasma levels as well as the release of VEGF in the supernatants of cell cultures from representative cases. Our aims were to evaluate whether abnormalities in the expression of this factor were associated with relevant laboratory or clinical findings and to define their possible prognostic value; moreover, to investigate a possible correlation between VEGF expression levels and various biological parameters such as CEC levels, BM microvessel density, apoptosis, proliferation.

Patients and methods

Patients

We analyzed BM cells from 188 patients with MDS not previously treated, stratified according to their BM blast percentages: 140 patients had less than 5% BM blasts (MDS-1) and 48 patients at least 5% BM blasts (MDS-2). Controls were 96 non-hemopathic subjects, gender- and age-matched; they presented normal blood count and none of them had evidence of underlying BM disease. In all cases BM aspiration had been performed as part of the diagnostic procedures. The study was approved by the Local Ethics Committee and it was in accordance with the Helsinki Declaration of 1975; informed consent was obtained from patients and controls.

MDS diagnosis was made according to the WHO classification.[16] There were 28 patients with refractory anemia (RA), 16 with RA with ring sideroblasts (RARS), 80 with refractory cytopenia with multilineage dysplasia (RCMD), 13 with MDS del(5q), 3 with MDS unclassifiable (MDS-U), 28 with RA with excess of blasts type 1 (RAEB 1) and 20 with RAEB 2.

The International Prognostic Scoring System (IPSS) was employed to define prognosis according to Greenberg et al. [17]; it could be assessed in 145 patients: 45 were classified at low risk, 69 as intermediate 1, 29 as intermediate 2, and 2 as high risk.

The clinical and haematological characteristics of the patients are summarized in Table 1.

Table 1. Clinical and hematological characteristics of the patients.

Variable	MDS-1 ($n = 140$)	MDS-2 ($n = 48$)
Age [years; median (IQR)]	68 (61.4–76)	69 (62.7–75)
Sex (M/F)	73/67	34/14
Hb [g/dL; median (IQR)]	9.3 (8.2–10.7)	9.0 (8.1–10.2)
WBC [$\times 10^9$/L; median (IQR)]	4.6 (3.0–6.3)	3.0 (1.9–5.4)
Neutrophils [$\times 10^9$/L; median (IQR)]	2.2 (1.5–3.2)	1.4 (0.8–2.4)
Platelets [$\times 10^9$/L; median (IQR)]	148 (80–254)	75 (32–100)
BM blasts [%; median (IQR)]	2 (1–3)	8 (6–11)
Karyotype (available)	114	31
Normal	59 (51.7%)	18 (58.0%)
Abnormal	55 (48.3%)	13 (42.0%)

CD34+ cell separation

BM mononuclear cells were separated using Lymphoprep (Axis-Shield, Oslo, Norway) density gradient centrifugation. CD34+ cells were isolated using the MiniMac system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol for separation. Following positive selection, CD34+ cells (>90% purity) were cytocentrifuged.

BM cell cultures

Mononuclear cells obtained by density gradient centrifugation from BM samples were resuspended in serum-free medium (X-vivo 15, Cambrex, Walkersville, MD) at a final concentration of 1.5×10^6/ml, and *in vitro* cultured in 24-well plates in a fully humidified atmosphere at 37 °C, 5% CO_2. After 96 h of incubation, supernatants were collected and centrifuged at 1200 rpm for 10 min and then stored at −20 °C.

BM plasma

BM plasma was separated from BM aspirates by centrifugation at 3000 rpm for 10 min and then stored at −20 °C.

VEGF detection

Immunocytochemistry

VEGF was detected on BM smears from all cases and controls and on CD34+ cell cytospins by an immunoalkaline phosphatase method using a primary murine monoclonal antibody raised against human VEGF (clone JH121). Negative controls were performed by replacing the primary antibody with non-immune mouse serum. In each case, the percentage of positive erythroid cells and the percentage of positive myeloid cells were recorded. Early myeloid precursors were identified on the basis of nuclear features (relatively large size and open chromatin pattern, with prominent nucleoli and/or irregular contour), which distinguished them from erythroid precursors.

VEGF release

VEGF levels were measured in the supernatants of BM cell cultures and in the BM plasma by a quantitative

sandwich enzyme immunoassay technique using a commercial kit (Quantikine Human VEGF, R&D Systems, Minneapolis, MN), according to the manufacturer's instructions.

Apoptosis

Apoptosis was measured by means of nuclear DNA fragmentation assay, using a terminal-deoxynucleotidyl-transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) technique. An alkaline phosphatase anti-fluorescein antibody was then used. The 'In situ Cell Death Detection Kit' (Roche Diagnostics GmbH, Mannheim, Germany) was used according to the manufacturer' instructions. The apoptotic index (AI) was expressed as the percentage of positive nuclei for at least 500 counted cells at a magnification of 1000×.

Proliferative activity

Cell proliferative activity was evaluated by an indirect immuno-alkaline phosphatase method (UltraVision Detection System Anti-Polyvalent, Alk-Phos/Fast Red, Lab Vision Corporation) using a murine monoclonal antibody to the nuclear antigen Ki-67 (MIB-1, Dakopatts, Glostrup, Denmark), expressed by cycling cells, on acetone fixed smears. In each sample, the percentage of cells with nuclear positivity was recorded.

Circulating endothelial cells

To evaluate CECs, peripheral blood cells phenotype was analyzed by flow cytometry using the following monoclonal antibodies: fluorescein isothiocyanate (FITC) anti-CD146 (Chemicon International, Milan, Italy); phycoerythrin (PE) anti-CD34 and anti-CD146 (Becton Dickinson, Milan, Italy); PE-Texas Red (ECD) anti-CD34 and anti-CD45 (Coulter-Instrumentation Laboratory, Milan, Italy) and PE-cyanine 5 (PC5) anti-CD45 (Coulter). Isotype-matched negative controls were used in all assays. Endotelial cells were identified in CD45− gate as CD146+/CD34 + cells. Multiparametric 4-color flow cytometry acquisition was performed with a FACSCanto flow cytometer (Becton Dickinson). Data, collected in list mode, were analyzed using the DIVA software (Becton Dickinson).

Marrow microvessel density

Microvessel density (MVD) was assessed in MDS patients as well as in marrows from controls as previously reported.[18] Briefly, the 3 μm thick paraffin sections obtained from trephine BM biopsies were immunostained with CD34 monoclonal antibody (clone: QB-End/10, Ylem, 1:100, pretreatment with citrate buffer 0.1 M, pH 6, in microwave oven, 2 cycles of 5 min at 400 W), using a Dako automated immunostainer (streptavidin-biotin-peroxidase method, chromogen: 3,3′-diaminobenzidine tetrahydrochloride). The substitution of primary antibody with nonimmune mouse serum was used as negative control. Vascular structures were defined as groups of CD34 + endothelial cells forming a structure with a discernible lumen. In each case, the absolute number of CD34 + vascular structures was recorded in 10 randomly selected fields at 400× magnification; arterioles with media layer were excluded from the count, while sinusoids were included.

Endothelial cell colonies

Endothelial colony-forming cells (ECFC) were assessed according to Asahara et al. [19], as reported by Della Porta et al. [18]. The endothelial nature of the colonies was confirmed by staining with anti-CD31 (Immunothech, Marseille, France), anti-CD144, anti-CD146, anti-von Willebrand factor (Dako) and anti-CD45 (Becton Dickinson) monoclonal antibodies. Colonies were counted using an inverted microscope, and their frequency was expressed as number of ECFCs per 10^7 mononuclear cells.

Statistical analysis

Descriptive statistics were computed as median and interquartile range (IQR) for continuous variables, due to their skewed distribution, or mean and SD; absolute and relative frequencies were computed for categorical variables. The values of the biological parameters were compared across diagnostic groups in MDS by means of generalized linear regression models. Spearman rank order R was used to assess any correlation between biological parameters. To verify whether VEGF expression levels did correlate with overall survival (OS) and progression-free survival (PFS) this variable was dichotomized according to a cutoff value identified by ROC analysis. Kaplan–Meier estimates of OS and PFS were computed and curves were compared using the log-rank test. Patients dying without previous evolution were censored at time of death. Multivariate analysis was performed according to the Cox's proportional hazard model. All tests were two-sided. A p value of $<.05$ was considered statistically significant. MEDCALC® 9.4.2.0 software was used for computation.

Results

VEGF expression

VEGF was detected in most maturing myeloid cells from normal samples (median 21%, IQR 9–29%), and in very rare normal erythroid precursors (median 0%, IQR 0–2%). In MDS VEGF myeloid levels (median 44%, IQR 31–56%) were higher than in controls ($p < .0001$), and also many erythroblasts expressed VEGF (median 23%, IQR 3–60%) ($p < .0001$), without significant difference between early and late forms of MDS or among WHO subtypes (Table 2). Positive cells showed strong diffuse cytoplasmic reactivity (Figure 1(A,B)).

VEGF expression was evaluated also in CD34 + cells isolated from BM mononuclear cells of 5 controls and 10 MDS patients (8 MDS-1 and 2 MDS-2). A few MDS CD34 + stem cells (2–16%) expressed VEGF, whereas normal CD34 + cells did not express this factor (data not shown).

VEGF release

The release of VEGF was demonstrated in all examined samples (6 controls, 11 MDS-1 and 2 MDS-2 cases), but VEGF levels trended to be higher in the media conditioned by MDS mononuclear cells from low-risk patients (median 56.3 pg/L, IQR 1.8–80.2) than in controls (median 9.4 pg/mL, IQR 0–22.8) ($p = .09$) (Table 3).

VEGF levels were evaluated in BM plasma from BM aspirates of 13 controls and 45 MDS patients (33 MDS-1 and 12 MDS-2); they were significantly higher in MDS BM plasma from patients without excess blasts (median 110.2 pg/mL, IQR 27.6–212.1) than in normal marrow plasma (median 15.4 pg/mL, IQR 7.7–71.8) ($p = .0111$), whereas there was no difference between normal marrow plasma levels and levels in cases with excess blasts (median 46.2 pg/mL, IQR 17–151.3) ($p = .21$) (Table 3).

MVD and CEC

BM MVD was significantly increased in the MDS-1 group (median 7.9, IQR 6.6–10.9) in comparison with the controls (median 4.7, IQR 4.4–6.1) ($p = .0009$) and the MDS-2 group (median 6.2, IQR 5.0–9.5) ($p = .0382$) (Figures 1(C,D) and 2(A)).

CEC levels were significantly higher both in MDS-1 (median 5.9×10^9/L, IQR 4.1–7.9) and in MDS-2 (median 3.4×10^9/L, IQR 2.7–5.1) than in controls (median 1.5×10^9/L, IQR 1.2–2) ($p < .0001$) (Figure 2(B)). ECFCs, defined by the endothelial progenitor cell (EPC) colony assay, were more numerous in MDS (median frequency per 10^7 mononuclear cells 0.5, range 0–0.76) than in controls (median 0.05, range 0–0.59) ($p = .03$) (data not shown).

In MDS, there was a positive correlation between MVD and CECs ($R = 0.47$, $p = .0015$), whereas no significant relationship was detected between VEGF expression and CEC levels ($R = 0.34$, $p = .34$) or MVD ($R = 0.02$, $p = .90$).

Other biological features

The AI ranged from 3% to 14% (median 8%) of the erythroid cells and from 13% to 37.8% (median 22%) of the myeloid cells in normal controls. In early MDS, the erythroid AI was significantly higher (median 15%, IQR 7–32.3%) than in normal controls ($p < .0085$) and trended to be higher than in advanced MDS

Table 2. VEGF expression, apoptosis and proliferation in BM cells. Values are expressed as median (IQR).

	VEGF-positive cells %					
Category	Total	*p* Value	Erythroid	*p* Value	Myeloid	*p* Value
Controls	16.5 (9–29)		0 (0–2)		21 (9–29)	
MDS	39 (27–52)	<.0001*	23 (3–60)	<.0001*	44 (31–56)	<.0001*
MDS-1	39 (28–52)	<.0001*	20 (3–60)	<.0001*	44 (31–57)	<.0001*
MDS-2	39 (21–55)	<.0001*	53 (13–73)	<.0001*	46 (30–63)	.0005*
	Apoptotic index (%)					
	Total	*p* Value	Erythroid	*p* Value	Myeloid	*p* Value
Controls	14 (11–23.5)		8 (3–14)		22 (13–37.8)	
MDS	26 (10–41)	.0186*	11 (7–22)	.0512*	35 (16.5–48)	.0186*
MDS-1	28 (20–41)	.0003*	15 (7–32.3)	.0085*	35 (17–51)	.0145*
MDS-2	24 (13–28.5)	.13*	9 (5.5–15)	.69*	35 (14–41)	.14*
	Proliferative activity (%)					
	Total	*p* Value	Erythroid	*p* Value	Myeloid	*p* Value
Controls	23 (19–26)		42 (25–53)		16 (14–20.5)	
MDS	25 (18–34)	.37*	31 (15.3–52)	.08*	17 (11.3–28.3)	.88*
MDS-1	25 (18.3–36.3)	.16*	34 (17–51.5)	.13*	16 (10–29)	.94*
MDS-2	20.5 (14–31.5)	.75*	27 (8.5–53.5)	.10*	19 (11.8–27.5)	.79*

*versus controls.

(median 9%, IQR 5.5–15%) ($p = .07$), whereas the myeloid AI was significantly higher in the whole MDS group (median 35%, IQR 16.5–48%) than in normal controls ($p = .0186$), without significant difference between early and late forms (Table 2).

In normal controls, the median percentages of 42% (IQR 24.8–53.3%) of the erythroid and 16% (IQR 14–20.5%) of the myeloid cells showed proliferative

Table 3. VEGF release.

Category	VEGF plasma levels pg/ml median (IQR)	*p* Value	VEGF cell culture supernate levels pg/ml median (IQR)	*p* Value
Controls	15.4 (7.7–71.8)		9.4 (0–22.8)	
MDS	29 (0.2–77.5)	.0178*	30 (0–65.8)	.14*
MDS-1	110.2 (27.6–212.1)	.0111*	56.3 (1.8–80.2)	.09*
MDS-2	46.2 (17–151.3)	.21*	4.4 (0.3–8.5)	.99*

*versus controls.

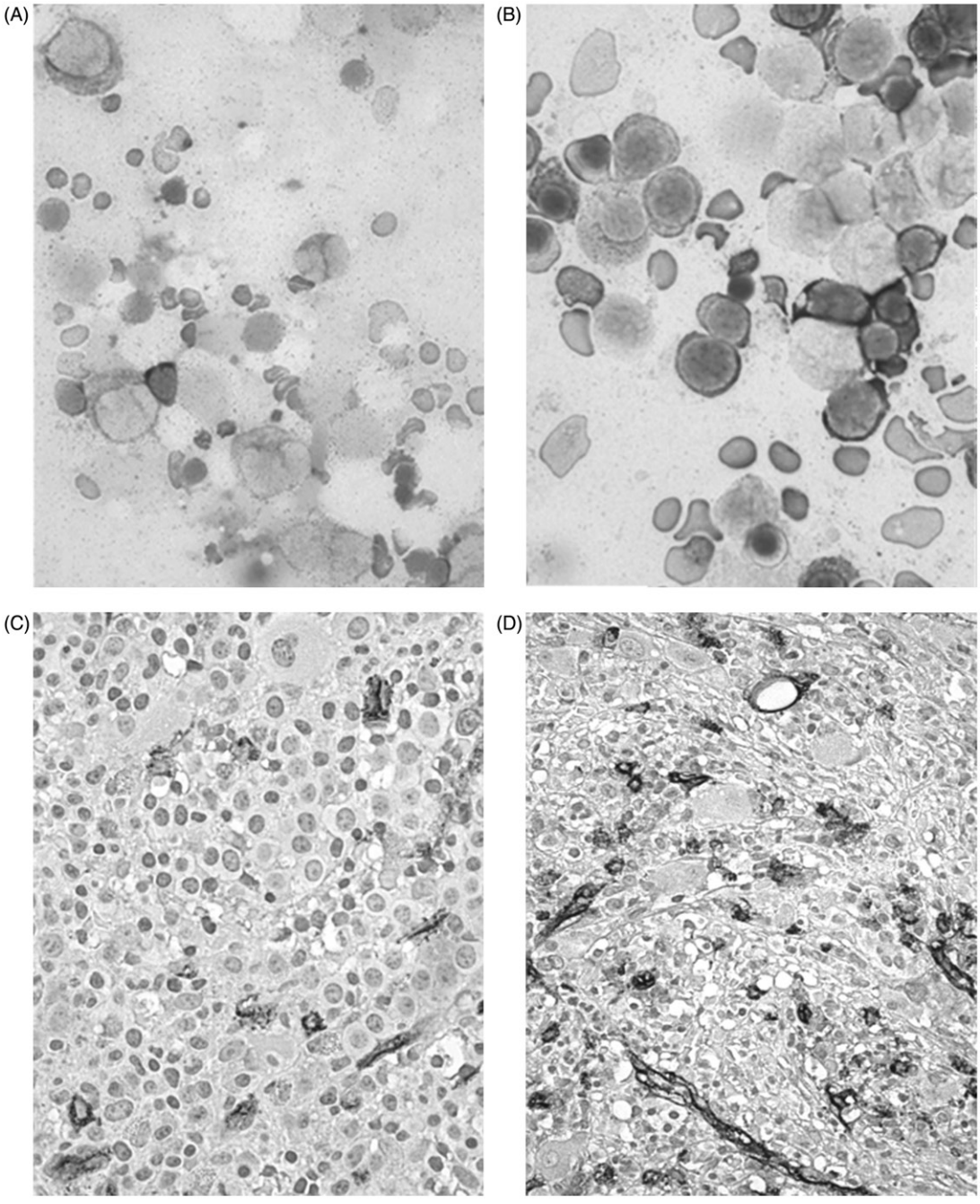

Figure 1. (A, B) Detection of VEGF in BM cells by an immunoalkaline phosphatase method (1250×). (A) Diffuse cytoplasmic positivity in myeloid cells and rare erythroblasts from a non-hemopathic subject. (B) In this BM sample from a RARS case erythroblasts show strong diffuse cytoplasmic expression of VEGF. (C, D) BM microvessel density assessed on trephine BM biopsies by CD34 monoclonal antibody immunostaining (400×). Low microvessel density in a control case (C) and a very high number of vascular structures in a RA case (D).

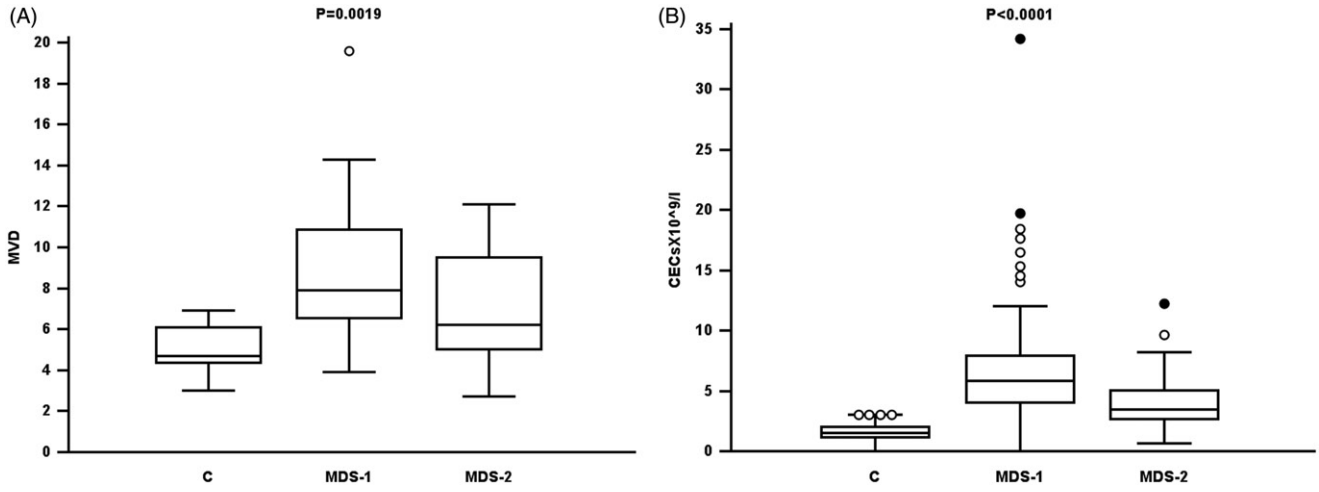

Figure 2. BM microvessel density (MVD) (A) and circulating endothelial cells (CEC) (B) in the different diagnostic subgroups. The control group is indicated as 'c'. Data are shown as median values, lower and upper quartiles, minimum and maximum values. 'Outside' and 'far out' values are displayed as separate points (empty dots and full dots, respectively).

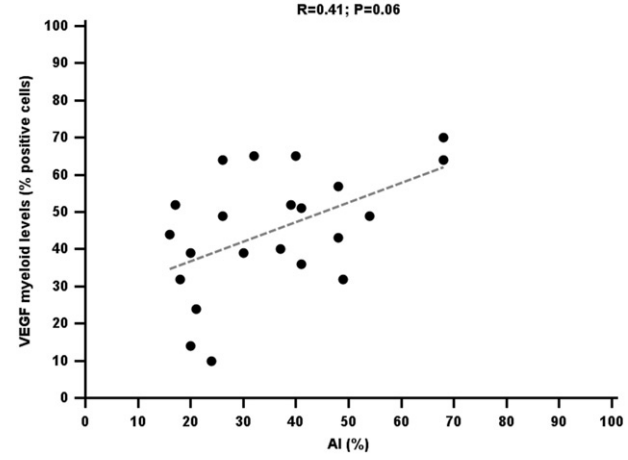

Figure 3. Relationship between percentages of VEGF positive myeloid cells and apoptotic index (AI) in MDS.

activity. Erythroid and myeloid proliferative activity did not differ significantly in early (median 34%, IQR 17–51.5% and 16%, IQR 10–29%, respectively) and advanced (median 27%, IQR 8.5–53.5% and 19%, IQR 11.8–27.5%, respectively) MDS (Table 2).

In MDS a trend to a positive correlation between VEGF myeloid levels and the total apoptotic rate ($R = 0.41$, $p = .06$) was observed (Figure 3), whereas VEGF expression was independent of the proliferative rate ($R = 0.01$, $p = .98$).

Correlation between VEGF expression and clinical and laboratory features in MDS

No significant relationship was observed between VEGF expression and clinical and laboratory features such as age, sex, hemoglobin level, platelet or leukocyte count, karyotype (data not shown).

MDS-1 patients were treated with supportive therapy or erythropoiesis stimulating agents whereas most MDS-2 patients were treated with hypomethylating agents. At the time of last follow-up, 33 patients (18%), 17 with MDS-1 and 16 with MDS-2, had died. In 57 cases (30%), 37 MDS-1 and 20 MDS-2, evolution toward a higher risk form or toward acute leukemia occurred after a median time of 13.2 months (IQR 4.1–23.9 months) following diagnosis. The median survival for all patients was 80.1 months, whereas the median PFS was 39.6 months. The prognostic value of IPSS variables was demonstrated ($p = .0001$ for OS and $p < .0001$ for PFS).

A cutoff value of 26% VEGF positive BM myeloid cells was able to discriminate MDS patients from control subjects, according to a ROC analysis (AUC 0.759, $p = .0001$). Kaplan–Meier estimates showed that in the whole MDS group a VEGF expression higher than 26% in myeloid cells was associated with significantly longer OS ($p < .0001$) (cumulative probability of survival 74% at 96 months versus 40%) and longer PFS ($p = .0002$) (cumulative probability of PFS 58% at 96 months versus 22%) (Figure 4). Even in the MDS-1 group alone, a VEGF expression higher than 26% in myeloid cells was associated with significantly longer OS ($p = .0201$) and longer PFS ($p = .0067$). In multivariate analysis, VEGF myeloid levels were predictors of survival independently of IPSS variables (Table 4). Multivariate analysis repeated with IPSS alone confirmed the independent prognostic significance of VEGF for OS

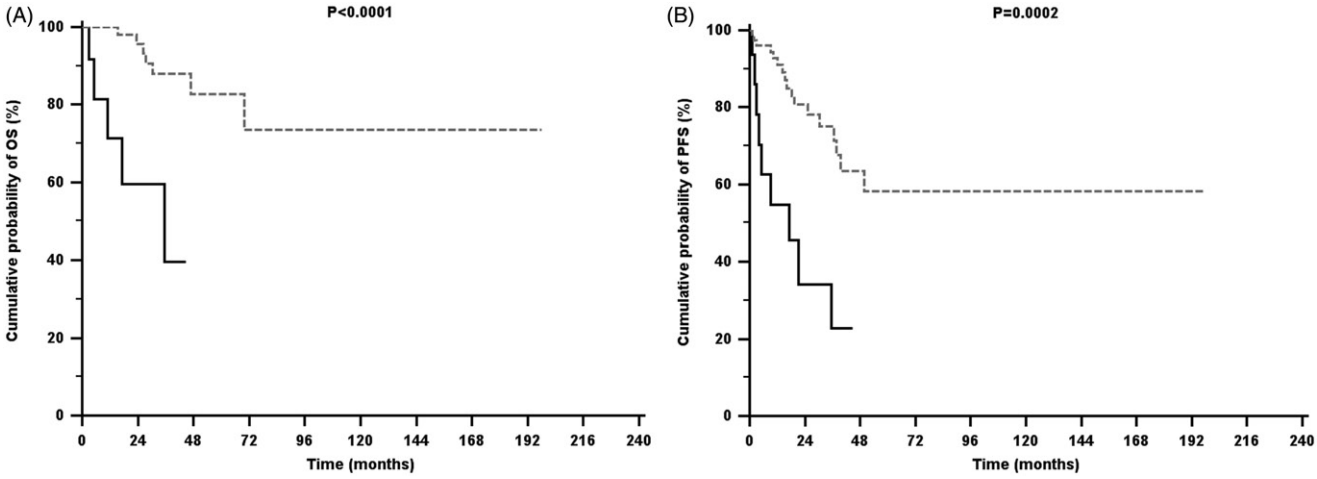

Figure 4. Kaplan–Meier curves of overall survival (OS) (A) and progression-free survival (PFS) (B) of patients with MDS according to VEGF expression in myeloid cells. A VEGF expression higher than 26% [160 cases (85%), dashed line] was associated with significantly longer OS and PFS than a low VEGF expression [28 cases (15%), continuous line].

Table 4. Multivariate analysis of overall survival and progression-free survival predictive factors in MDS patients (Cox proportional hazard model).

	Overall survival			Progression-free survival		
Variable	Hazard ratio	95% CI	*p*	Hazard ratio	95% CI	*p*
BM blasts (%)	1.5	1.13–2.00	.0060	1.19	1.00–1.40	.0447
Cytogenetic risk (good-intermediate; poor)	8.41	1.79–39.55	.0073	3.35	1.59–7.08	.0016
Cytopenia (1;2;3)	3.74	1.51–9.29	.0046	1.70	0.99–2.92	.0539
VEGF levels (positive myeloid cells %)	0.92	0.87–0.98	.0064	0.95	0.92–0.98	.0029

(HR 0.95, 95%CI 0.91–1, $p = .0024$) and PFS (HR 0.96, 95%CI 0.93–0.99, $p = .0007$).

Discussion

The expression of various angiogenesis mediators has been found to be altered in MDS BM and abnormal angiogenesis has been implicated in the pathogenesis of the disorder.[2,3] Although the precise role of neoangiogenesis in the development and progression of MDS is not clear, many authors agree on the usefulness of its evaluation for diagnostic and/or prognostic purpose even by using different methodological approaches.[1,12–14,19,20]

However, the studies concerning clinical implication of VEGF expression in MDS are limited and its prognostic significance remains controversial. Recently, Savic et al. [15] demonstrated the negative prognostic significance of an increased MVD in MDS, whereas VEGF levels did not influence prognosis. On the contrary, Gianelli et al. [21] found high immunohistochemical VEGF expression levels in BM biopsies from patiens with high-risk MDS and showed the predictive role of this factor for transfusion dependence as well as for shorter leukemia-free survival and OS.

In our study, we have confirmed an abnormal expression of VEGF in MDS and evaluated its relation with various biological and clinical findings.

In agreement with other authors,[10,14] we observed that, in normal controls, CD34 + progenitors did not express VEGF, whereas various percentages of maturing myeloid cells contained the growth factor in their cytoplasm. In MDS BM, VEGF was present both in CD34 + cells and in myeloid cells at different maturation stages. Also many myelodysplastic erythroblasts, at any maturation stage, were VEGF positive differently from normal erythroblasts. Moreover, BM cells proved able to release the growth factor.

While other authors reported higher VEGF expression levels in advanced MDS, we found significantly increased VEGF expression and release also in the MDS subgroups without excess blasts. In addition, we observed pronounced abnormalities of other angiogenesis parameters such as MVD and CECs especially in low-risk MDS. These results, confirming our previous findings,[18] strengthen the suggestion that, in MDS, the generation of new vessels is mandatory to provide an adequate microvascular network for the multistep process of conversion from normal to dysplastic BM.

In MDS, however, the production and release of VEGF, besides stimulating angiogenesis, might alter the BM microenvironment and the interaction between hematopoietic progenitors and stromal cells, and so influence cell survival and differentiation.[22–24] A well-known hallmark of MDS is the excessive intramedullary death of the most mature hematopoietic precursors, leading to ineffective hematopoiesis.[25] Presumably, VEGF released in excess by MDS BM cells, after interaction with factors in both the cytoplasm and nucleus and up- or down-regulation of the expression of various genes, stimulates endothelial cells as well as macrophages to produce inflammatory cytokines that can induce erythroid or myeloid apoptosis and, as a consequence, ineffective erythropoiesis or granulopoiesis.[26,27] Indeed, our finding of a trend to a correlation between VEGF myeloid levels and apoptotic rate is in agreement with this hypothesis. On the other hand, a few years ago, it was shown that neutralization of VEGF activity suppressed the generation of interleukins as TNF-α and IL1b from MDS mononuclear cells and BM stroma and promoted the formation of CFU-GEMM and BFU-E in methylcellulose cultures.[11]

This possible pathogenetic mechanism could partially explain another important issue, i.e. the independent favorable prognostic significance of high VEGF myeloid expression in MDS. We assume that the specific alterations of BM microenvironment induced by the cytokine network under the influence of VEGF might determine an imbalance of the cell proliferation-death ratio in favor of apoptosis and reduce the risk of disease progression. On the contrary, other authors observed that, in solid or hematologic tumors, high VEGF levels were negative prognostic predictors, promoting tumor spreading and metastatization.[28,29] Some of the discrepancies with respect to our data could be explained by the different biological characteristics of solid tumors and acute leukemias compared to MDS and also by the smaller size of the groups of MDS patients previously studied. Anyway, an important limitation of our study was the heterogeneous treatment of the patients; for this reason, any conclusion must be drawn with caution and our findings should be validated by further studies with larger patient populations, more homogeneous treatment and longer follow-up. The precise mechanism of the increased expression of VEGF in MDS cells has not yet been identified. Finding the molecular mechanisms that underlie VEGF overexpression could provide important contributions from the therapeutic point of view.[30] VEGF deregulated expression in MDS may already constitute a potential target for experimental treatments. Promising results were obtained by the MDS French Group, who used the anti-VEGF antibody Bevacizumab for the treatment of patients with MDS with excess blasts.[31] A significant reduction of BM MVD as well as of VEGF circulating levels were observed, but only one case achieved a clinical response with complete transfusion independence. This is, however, the first demonstration of the possible efficacy of an anti-VEGF agent in this disease, with the encouraging prospect of modifying patients survival, besides quality of life. A more recent study demonstrated that a VEGF receptor tyrosine kinase inhibitor, Vatalinib, was able to induce improvement in blood count in only a small proportion of MDS patients and clinical applicability was limited by side effects [32,33]; also the small molecule receptor tyrosine kinase inhibitor sunitinib was not well tolerated by patients with MDS, chronic myelomonocytic leukemia and AML, most of which came off study without completing one cycle of therapy.[34] Moreover, no confirmed responses were observed in patients with AML or high-risk MDS treated with cediranib, that is another VEGF receptor inhibitor,[35] whereas preliminary data showed activity of ilorasertib, a dual Aurora/VEGF receptor inhibitor, in a few patients with myeloid malignancies.[36] On the basis of these results, the opportunity to use anti-VEGF agents in combination with other drugs potentially effective in MDS was suggested.[35]

Our observation, also in low-risk MDS, of high VEGF levels, that trended to be associated with higher apoptosis rates, suggests a probably best suited use of anti-VEGF drugs for the treatment of low-risk forms, in order to reduce ineffective hematopoiesis improving blood counts, rather than inhibiting leukemic progression.

In conclusion, our data confirm the importance of the dysfunction of the BM microenvironment in the pathogenesis of MDS and underscore the need to restore the normal interactions between hematopoietic progenitors and microevironment for therapeutic purposes.

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