Evaluation of *VEGF* and *VEGFR* gene expression as prognostic markers in low and intermediate-1 risk patients with myelodysplastic syndromes

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Abstract. Vascular endothelial growth factors (VEGFs) are angiogenic factors playing a key role in tumor development. VEGFs are produced by different normal and tumor cells, including platelets, lymphocytes and mononuclear cells of peripheral blood. *VEGF* (*VEGF-A*, *VEGF-C* and *VEGF-D*) and *VEGFR* (*VEGFR1*, *VEGFR2* and *VEGFR3*) gene expression was studied in patients with myelodysplastic syndrome (MDS) to evaluate the possible prognostic role of the expression of these genes. Gene expression levels were determined using peripheral blood samples of 51 patients with MDS and 15 healthy volunteers by quantitative PCR. Expression of all *VEGF* and *VEGFR* genes was elevated in patients with MDS compared with healthy volunteers. No association of *VEGF-A* expression with the hemoglobin content in peripheral blood was found. The analyses of gene expression in patients with MDS stratified by risk groups according to the International Prognostic Scoring System showed progressive augmentation of *VEGF-A* gene expression from low to high-risk groups and *VEGFR1* and *VEGFR2* expression from intermediate-1 to high-risk groups. The statistically significant difference in survival time of patients with high and low levels of *VEGFR1* expression was revealed. *VEGF-A*/*VEGFR1* expression may be important for risk evaluation of patients with MDS.

Introduction

Myelodysplastic syndromes (MDSs) are a heterogeneous group of blood disorders characterized by peripheral blood cytopenia due to ineffective hematopoiesis, dysplasia in ≥1 hematopoietic cell lineages and increased risk of transformation to acute myeloid leukemia (AML). MDSs are hypothesized to be clonal stem cell disorders arising from accumulation of multiple gene abnormalities, such as somatic point mutations, copy-number alterations and chromosomal aberrations (1). Genomic and chromosomal instability and variable molecular mechanisms contribute to pathogenesis and prognosis of MDS (2).

Accumulation of bone marrow (BM) blasts is a key feature and one of the main risk criterion and prognostic factors in patients with MDS (3). However, the majority of patients with MDS die from causes intrinsic to the disease, such as infection, pneumonia, sepsis and bleeding, which are not associated with leukemic transformation (4). This is particularly relevant to patients with lower risk MDS, which is defined as low or intermediate-1-risk according to the International Prognostic Scoring System (IPSS) (5).

VEGFs and their receptors, VEGFRs, are involved in regulation of proliferation, migration, invasion and differentiation of normal and cancer cells (6-9). VEGFs exert their effects by binding to receptors of the VEGFR family that consists of tyrosine kinase receptors, VEGFR1, VEGFR2 and VEGFR3 (10). The role of VEGF-A/VEGFR1 and VEGF-A/VEGFR2 signaling pathways has been evaluated in certain hematological malignancies, such as multiple myeloma, lymphoma and myeloproliferative neoplasms (11-13). However, a potential role of VEGF-A-dependent signaling in MDS pathogenesis has been poorly studied and the results are contradictory. Studies have demonstrated that BM blasts express VEGF-A and modulate VEGF-A-dependent autocrine loop signaling in MDS (14,15). However, Aguayo *et al* (16) found no prognostic significance of plasma VEGF-A levels in patients with MDS but suggested that VEGF-A plays a role as a prognostic factor in patients with AML. Verstovsek *et al* (17) showed that increased VEGF-A expression in BM specimens is inversely associated with survival time in patients with MDS or AML, whereas VEGFR1 and VEGFR2 expression levels have no prognostic impact.

To the best of our knowledge, there is no published data on the role of VEGF-C, VEGF-D ligands and VEGFR3 in

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MDS pathogenesis. However, a previous study revealed that activation of the VEGF-C/VEGFR3 pathway promotes cancer cell mobility to induce metastasis and an increase in VEGF-C and/or VEGFR3 expression may be associated with a shorter survival in numerous types of malignancy (18). The aim of the present study was to evaluate *VEGF* and *VEGFRs* expression as putative prognostic markers for MDS.

Patients and methods

Patients and controls. The study group consisted of 51 patients with verified MDS (31 female and 20 male) with a median age of 69.8 years (range, 59-77 years) who were diagnosed between January 1, 2011 and August 31, 2018 and treated at A.S. Loginov Moscow Clinical Scientific Center (Moscow, Russia). The diagnosis of MDS was based on cytological examination of peripheral blood cells. The patients were followed-up from MDS diagnosis until May 31, 2019. The transformation to AML or the death of patients was considered, if they occurred during the follow-up period. The control group consisted of 15 volunteers (8 females and 7 male) free of neoplasms or any other abnormality. The median age and range were 65.3 and 61-68 years, respectively. Clinical and hematological variables (hemoglobin content and platelet and leukocyte count) in the control group were within normal ranges.

Laboratory procedures. Hemoglobin concentration, as well as platelet and leukocyte counts were measured using the automated hematology analyzer ADVIA 2120i according to the manufacturer's recommendations (Siemens Healthineers AG).

Inclusion and exclusion criteria. Inclusion criteria were *de novo* female and male patients with MDS aged ≥18 years old or patients with MDS who only received prior supportive care (such as red blood cell and/or platelet transfusions for severe anemia and severe thrombocytopenia improvement). Patients treated with erythropoiesis-stimulating agents (in patients with chromosome 5q deletion) were also included. Patients with BM blast cells=5% were excluded. Patients previously treated with hypomethylating agents and/or who received immunosuppressive therapy were excluded. Patients with the hypoplastic variant of MDS as well as patients that refused to participate were not included.

The patient risk stratification according to the 2017 World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissue (19) and IPSS (20), as well as other patient characteristics are presented in Table I. Karyotype was classified using the International System for Human Cytogenetic Nomenclature (20,21).

Mononuclear cell preparation and cDNA synthesis. The peripheral blood specimens (5-10 ml) were separated using a Ficoll® density gradient (PanEko) and the obtained mononuclear cell fraction was used for further study. Total RNA was isolated by TRI Reagent® (Molecular Research Center). All procedures were as previously described (22). Briefly, cDNA synthesis reaction mixture contained 1 μg purified total RNA, 1 μl random 6 primers (Syntol), 2.5 mM dNTP mixture (Thermo Fisher Scientific, Inc.), 0.4 units RNase inhibitor (Thermo Fisher Scientific, Inc.) and 2 units M-MuLVplus reverse transcriptase (Thermo Fisher Scientific, Inc.). The mixture volume was 25 μl. The synthesis was performed in Terzic' thermocycler (DNA technology, Russia) at 42˚C for 50 min with pre-incubating for 10 min at 25˚C. The reaction was stopped by heating at 70˚C for 10 min.

Quantitative PCR (qPCR). The amplification of cDNA was performed in a Bio-Rad CFX (Bio-Rad Laboratories, Inc.) detection system using EvaGreen® dye (Biotium) and qPCR master mix (Syntol) according to the manufacture's protocol. PCR conditions for all genes were 95˚C for 5 min followed by 39 cycles of 95˚C for 20 sec, 59˚C for 25 sec and 72˚C for 20 sec. Each sample was measured in triplicate. For data standardization, the 60S subunit of the *RPL27* gene was used. The relative expression was determined according to the $2^{-\Delta\Delta Cq}$ equation [$\Delta C_q = C_q$ (*RPL27*)-C_q (test gene), where C_q is the threshold cycle of the gene in the exponential phase of the amplification curve] (23). The following primers were used: *VEGF-A* forward, 5'-AGGGCAGAATCATCACGAAGT-3' and reverse, 5'-AGGGCTTCGATTGGATGGCA-3'; *VEGF-C* forward, 5'-GAGGAGCAGTTACGGTCTGTG-3' and reverse, 5'-tcctttccttagctgacacttgt-3'; *VEGF-D* forward, 5'-TCCCATCGGTCCACTAGGTTT-3' and reverse, 5'-AGGGCTGCACTGAGTTCTTTG-3'; *VEGFR1* forward, 5'-TTTGCCTGAAATGGTGAGTAAGG-3' and reverse, 5'-TGGTTTGCTTGAGCTGTGTTC-3'; *VEGFR2* forward, 5'-GGCCCAATAATCAGAGTGGCA-3' and reverse, 5'-CCAGTGTCATTTCCGATCACTTT-3'; *VEGFR3* forward, 5'-TGCACGAGGTACATGCCAAC-3' and reverse, 5'-GCTGCTCAAAGTCTCTCACGAA-3' and *RPL27* forward, 5'-ACCGCTACCCCCGCAAAGTG-3' and reverse, 5'-CCCGTCGGGCCTTGCGTTTA-3'.

Statistical analysis. All qPCR experiments were performed in triplicate. Data are presented as the mean ± SEM. Correlation was analyzed using Pearson's rank test. Overall survival was estimated by Kaplan-Meier method with log-rank test. To assess diagnostic value of *VEGF* and *VEGFRs* gene expression as candidate biomarkers was performed receiver operating characteristic (ROC) analysis. Area under ROC curve (AUC) was used to compare the discriminatory performance of putative markers to determine their utility as a novel diagnostic test. Statistical significance was analyzed using an unpaired two-tailed Student's t test. $P<0.05$ was considered to indicate a statistically significant difference. All statistical calculations were performed using GraphPad Prism for Windows program, Version 5.00 (Trial), 2007 (Dotmatics).

Results

VEGF and VEGFR expression is elevated in patients with MDS. VEGF (*VEGF-A*, *VEGF-C* and *VEGF-D*) and *VEGFR* (*VEGFR1*, *VEGFR2* and *VEGFR3*) gene expression levels were studied in the peripheral blood samples of 51 patients with MDS and 15 healthy donors. Gene expression varied considerably in patients with MDS compared with healthy donors (Fig. 1A). Although no statistical difference between patients with MDS and controls in *VEGFR1* and *VEGFR3* expression was found, relative *VEGFR1* expression was $0.4\text{-}20.0\text{x}10^{-3}$ in patients with MDS and from $1.5\text{x}10^{-3}$ to

Table I. Clinical variables of 51 patients with MDS.

Clinical variable	Value
Median age (range), years	69.80 (59.00-77.00)
Sex, n	
Female	31.00
Male	20.00
WHO classification, n	
MDS-SLD	8.00
MDS-RS	2.00
MDS-MLD	11.00
MDS-EB[a]	22.00
MDS-del(5q)	8.00
IPSS classification, n	
Low	15.00
Intermediate-1	12.00
Intermediate-2	9.00
High	15.00
Karyotype[b], n	
Good	30.00
Intermediate	2.00
Poor	6.00
n/d	13.00
Mean hemoglobin count ± SEM, g/dl	6.59±1.60
Mean platelets count ± SEM, $x10^9$/l	118.33±60.85
Mean leukocyte count ± SEM, $x10^9$/l	4.67±3.53
Bone marrow blasts, n	
>5%	24.00
<5%	27.00
AML progression, n	14.00
Death, n	26.00
Survival time ± SD, months	24.80±22.68

[a]MDS-EB is EB1 + EB2. [b]Cytogenetic subgroups: Good, normal karyotype. Isolated-Y, del(5q) or del(20q); poor, ≥3 abnormalities or chromosome 7 anomalies; intermediate, other abnormalities; n/d, not determined; MDS-SLD, myelodysplastic syndrome with single-lineage dysplasia; MDS-RS, MDS with ring sideroblasts; MDS-MLD, MDS with multilineage dysplasia; MDS-EB, MDS with excess blasts; MDS-del(5q), MDS with isolated 5q-deletion; IPSS, International Prognosis Scoring System; AML, acute myeloid leukemia.

$4.2x10^{-3}$ in control group (mean, $2.71±0.46x10^{-3}$ vs. $2.82±0.19$) $x10^{-3}$. Similarly, *VEGFR3* relative expression varied from $0.1\text{-}8.0x10^{-3}$ in patients with MDS and from 0 to $3.4x10^{-3}$ in controls (mean values: $(1.78±0.24)$ $x10^{-3}$ vs. $1.02±0.30x10^{-3}$. *VEGF-A* and *VEGF-C* expression were higher in patients with MDS than in controls. The mean values of relative *VEGFA* expression in patients with MDS and in control group were $(19.73±2.84)$ $x10^{-3}$ and $11.07±0.99x10^{-3}$. The mean values of relative *VEGF-C* expression in MDS patients and control group were $(22.50±3.88)$ $x10^{-3}$ and $9.23±1.69x10^{-3}$. *VEGFR2* expression levels were low in the group of healthy volunteers (mean value 0.08±0.04) and upregulated in patients with MDS (mean value 0.82±1.39), indicating that VEGFR2-dependent signaling was stimulated in patients with MDS. *VEGF-D* expression was absent in control group and varied from 0 to $1.4x10^{-3}$ in patients with MDS.

VEGF-A is activated under hypoxic conditions (24). To evaluate the association between *VEGF-A* expression and hypoxia, levels of *VEGF-A* expression and hemoglobin content in the peripheral blood samples of patients with MDS was compared. No correlation was found between these two variables (Fig. 1B). Moreover, the mean value of hemoglobin was almost the same in patients with MDS with different levels of *VEGF-A* expression (above and below the mean level of $19.62x10^{-3}$ of relative *VEGF-A* gene expression; Fig. 1C). Thus, *VEGF-A* expression was not dependent on the content of hemoglobin in peripheral blood of patients with MDS.

To determine if there was an association between clinical characteristics of patients with MDS and gene expression, levels of hemoglobin, platelets and leukocytes were compared with *VEGF* and *VEGFR* gene expression levels. Significant associations were found between levels of platelets and the expression levels of *VEGF-C* and *VEGF-D* (Fig. 1D and E).

VEGF and VEGFR expression in patients with MDS with different risk of disease development. All patients with MDS were stratified in the present study, according to the IPSS. Patients were assigned to low (15 patients), intermediate-1 (12 patients), intermediate-2 (9 patients) and high (15 patients) risk groups and the survival time and gene expression in these groups were compared. No statistical difference in survival time between any of these risk groups was found. Nevertheless, the median survival time diminished from the intermediate-1 (51 months) and intermediate-2 (49 months) risk groups to the high-risk group (23 months). The median survival time in the low-risk group was not reached (Fig. 2A).

VEGF-A expression was progressively elevated from the low to the high-risk groups (Fig. 2). A statistically significant difference was found for *VEGF-A* expression between the low and intermediate-2 risk groups, as well as between the low and high-risk groups. No statistically significant difference between risk groups in *VEGF-C*, *VEGF-D*, *VEGFR1* and *VEGFR2* expression was found. Expression of *VEGFR1* and *VEGFR2* genes was notably elevated from the intermediate-1 to the high-risk group. *VEGF-C* and *VEGF-D* expression was notably higher in the low-risk group of patients compared with the other groups. *VEGFR3* expression was lowest in the high risk group. The only statistically significant difference in gene expression was found between the intermediate-2 and high risk groups for *VEGFR3*.

VEGF and VEGFR expression in patients with MDS with different levels of BM blasts. A key prognostic factor characterizing patients with MDS is the percentage of BM blast cells. BM blast cells <5% is usually associated with good prognosis (20). In the present study, all patients from low and intermediate-1 risk groups had BM blast levels <5% and patients from intermediate-2 and high-risk groups had BM blast levels >5%. The survival time and gene expression levels of MDS patients with BM blast levels <5% (27 patients) and >5% (24 patients) were compared. No significant difference in survival time between these patients was found, although

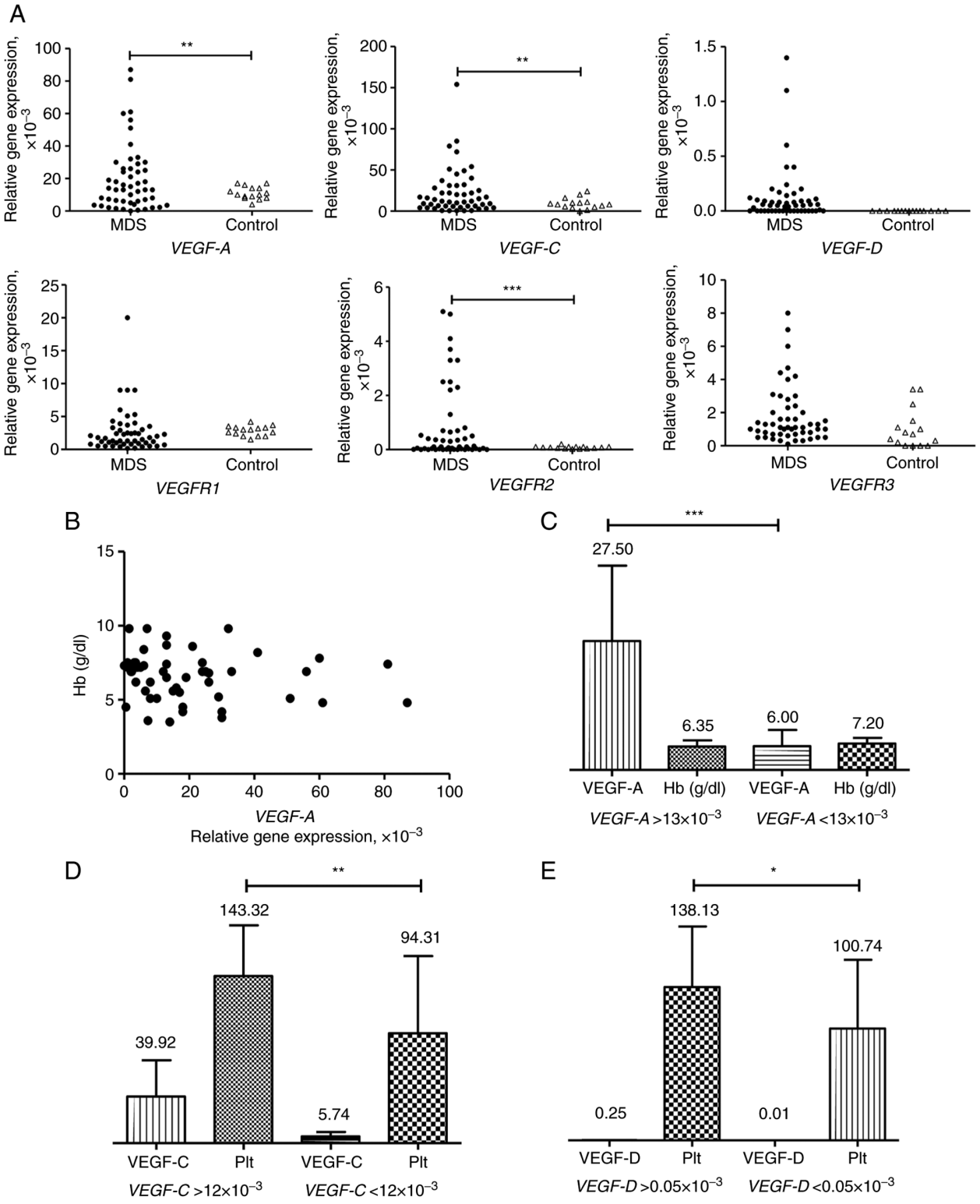

Figure 1. *VEGF* and *VEGFR* expression, the Hb content and Plt count in patients with MDS. (A) Relative *VEGF* and *VEGFRs* gene expression in patients with MDS and healthy controls using reverse transcription-quantitative PCR. (B) Distribution of patients with MDS according to *VEGF-A* expression and Hb content. (C) *VEGF-A* expression and Hb levels (g/dl) in patients with MDS with *VEGF-A* expression above or below its median level ($13.0x10^{-3}$). (D) *VEGF-C* expression and Plt ($x10^9$/l) levels in patients with MDS with *VEGF-C* expression above or below its median level ($12.0x10^{-3}$). (E) *VEGF-D* expression and Plt ($x10^9$/l) levels in patients with MDS with *VEGF-D* expression above or below its median level ($0.05x10^{-3}$). Data are presented as the mean ± SEM and were analyzed using an unpaired two-tailed Student's t-test. *P<0.05, **P<0.01, ***P<0.001. MDS, myelodysplastic syndrome; Hb, hemoglobin; Plt, platelet.

the median survival times were different (51 vs. 28 months for <5% and >5% BM blast levels, respectively; Fig. 3A).

Although there was no statistically significant difference, the mean *VEGF-A*, *VEGFR1* and *VEGFR2* expression levels were elevated in patients with MDS with >5% BM blast levels (Fig. 3B and C). *VEGF-C*, *VEGF-D* and *VEGFR3* expression was decreased in the group of patients with MDS with BM blast levels >5%.

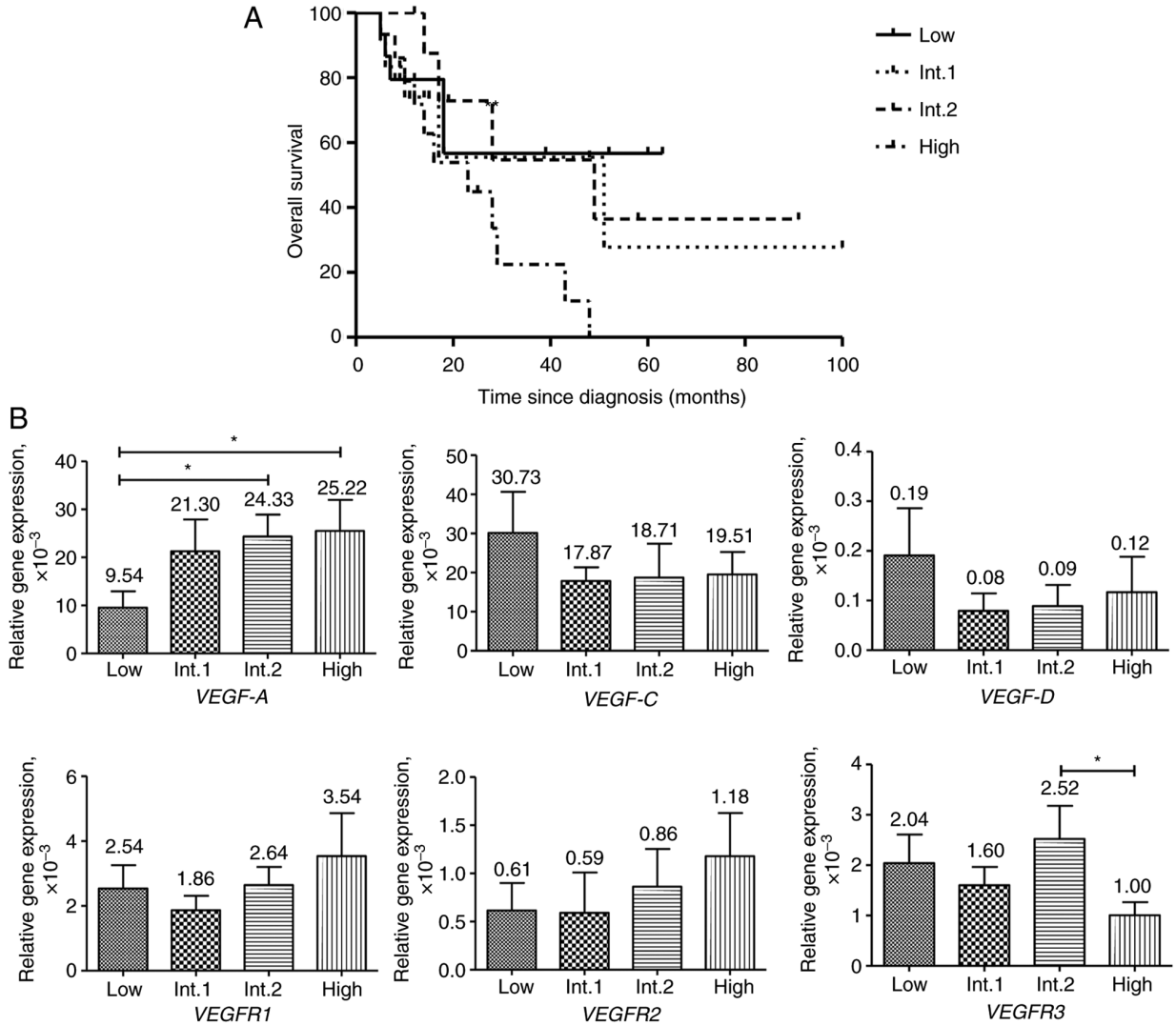

Figure 2. Survival and *VEGF* and *VEGFRs* expression in different groups of patients with MDS. (A) Overall survival in patients with MDS and low, Int.1, Int.2 and high-risk of disease development. (B) Relative *VEGF* and *VEGFRs* expression in MDS patients with low, Int.1, Int.2 and High-risk of disease development. Data are presented as the mean ± SEM and were analyzed using an unpaired two-tailed Student's t test. *P<0.05. Int, intermediate.

The prognostic evaluation of potential clinical outcomes in MDS patients with BM blast levels <5% is complicated. Some of these patients die within a few months of developing AML transformation or other complications of bone marrow failure, while others can survive for a long time (25). Survival time of patients with BM blast levels <5% (group 1, 9 patients) did not differ from that of patients with BM blast levels >5% (17 vs. 14 months, respectively), while patients of a group 2 with BM blast levels <5% had an improved survival (group 2, 18 patients; Fig. 3A). *VEGF* and *VEGFRs* gene expression levels in groups 1 and 2 were compared (Fig. 3D and E) and *VEGF-A*, *VEGF-C*, *VEGFR1* and *VEGFR2* expression in these groups was very similar to that in patients with MDS with BM blast levels >5 and <5% (Fig. 3B and C).

Survival of MDS patients with different VEGF-A and VEGFR1 expression levels. As both *VEGF-A* and *VEGFR1* gene expression levels were elevated in patients with MDS with a worse prognosis, the survival of patients was compared. No statistically significant difference was found between patients with MDS with high and low levels of *VEGF-A* expression, although the median survival times in patients with *VEGF-A* expression levels above (18 patients) and below (33 patients) the mean value ($1.96x10^{-3}$) were different (23 and 43 months, respectively; Fig. 4A). Patients with MDS with *VEGFR1* expression levels exceeding the mean relative *VEGFR1* gene expression ($2.69x10^{-3}$; 15 patients) had statistically worse survival (log-rank test; Fig. 4B) with a median survival time of 17 months compared with 48 months for the group with low levels of *VEGFR1* expression (36 patients).

To evaluate the diagnostic value of *VEGF-A* and *VEGFR1* gene expression levels as candidate biomarkers of MDS, receiver operating characteristic (ROC) curve analysis was applied. Area under the curve (AUC) was 0.57 for the *VEGF-A* gene, but the result was not statistically significant (Fig. 5A). However, *VEGFR1* expression levels discriminated between patients with MDS and the healthy controls (AUC=0.684), suggesting its potential diagnostic value (Fig. 5B).

Discussion

Due to the heterogeneity of MDSs at morphological, clinical and molecular levels, the accurate diagnosis of these diseases

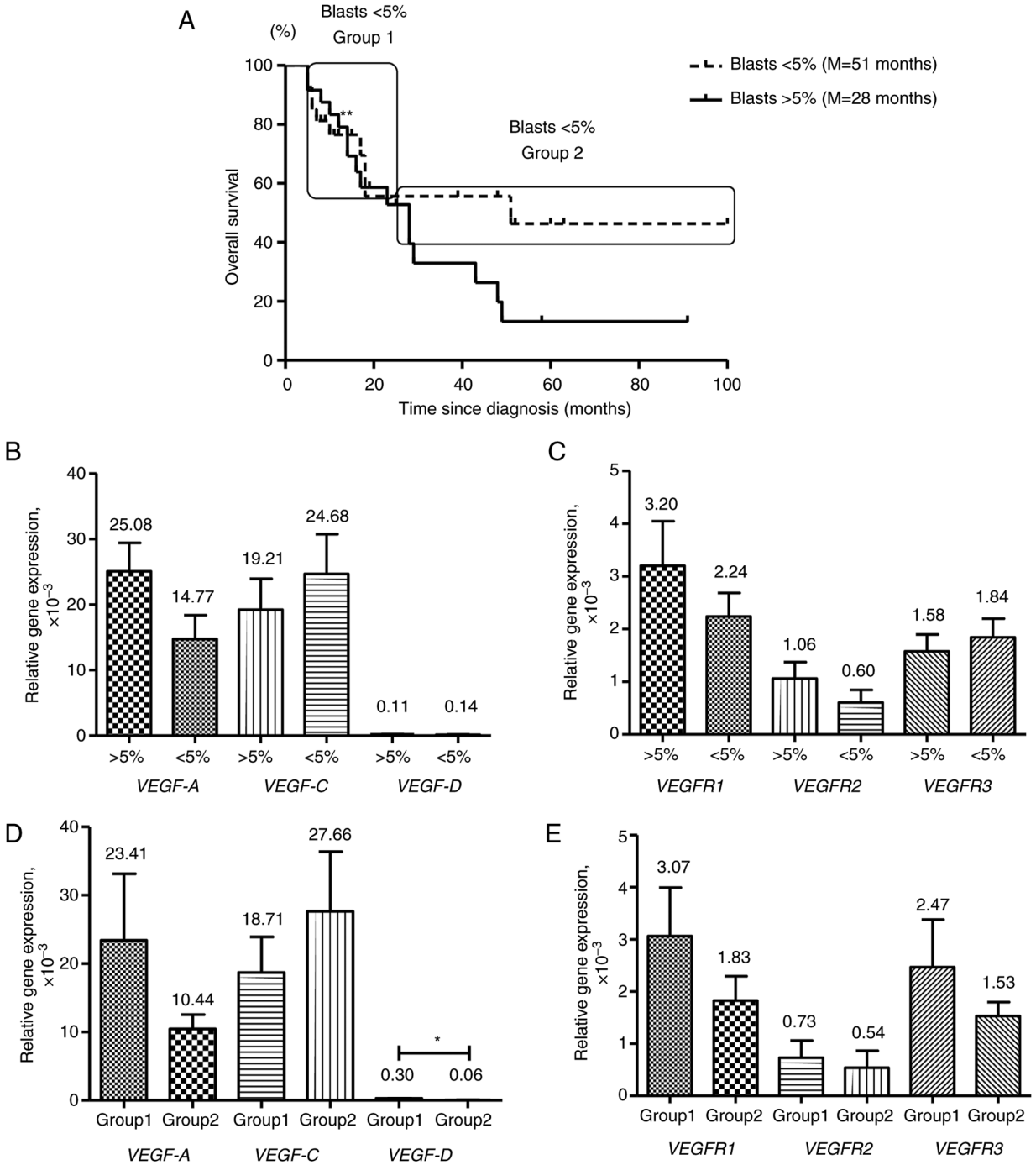

Figure 3. Survival and *VEGFs*, *VEGFR* expression in different groups of patients with MDS. (A) Kaplan-Meier survival curves of patients with MDS with differing levels of BM blasts; cut-off value of blasts was 5%. Patients with MDS with BM blast levels <5% were subdivided into groups of patients with a worse (group 1) and improved (group 2) survival rate. Relative (B) *VEGF* and (C) *VEGFR* gene expression in groups of patients with MDS with >5 or <5% BM blast levels. Relative (D) *VEGF* and (E) *VEGFR* expression in groups 1 and 2 of patients with MDS. Data are presented as mean ± SEM and were analyzed using an unpaired two-tailed Student's t test. *P<0.05. M, median; MDS, myelodysplastic syndrome.

has certain problems (subjectivity of morphological assessment of bone marrow aspirate and biopsy, as well as heterogeneity of cytogenetic alterations) and the expected clinical outcome for patients with MDS is different. Whereas some patients transform to AML or die from complications of BM transplant failure within a few months, other patients with MDS survive for years without major hematological problems (26). A precise diagnosis is important for the prediction of patient survival and risk of AML transformation and selection of appropriate therapy.

Several classification systems have been presented to evaluate MDS diagnosis, the most commonly used being the French-American-British (FAB) (19) and IPSS (20) classification. In the present study, patients with MDS were stratified according to IPSS scoring. The diagnosis of MDS is often based on morphological characteristics, and due to the subjectivity of this evaluation, discrepancy in MDS diagnoses exists. Naqvi *et al* (27) analyzed discordance between the diagnosis of 915 patients with MDS referred according to FAB

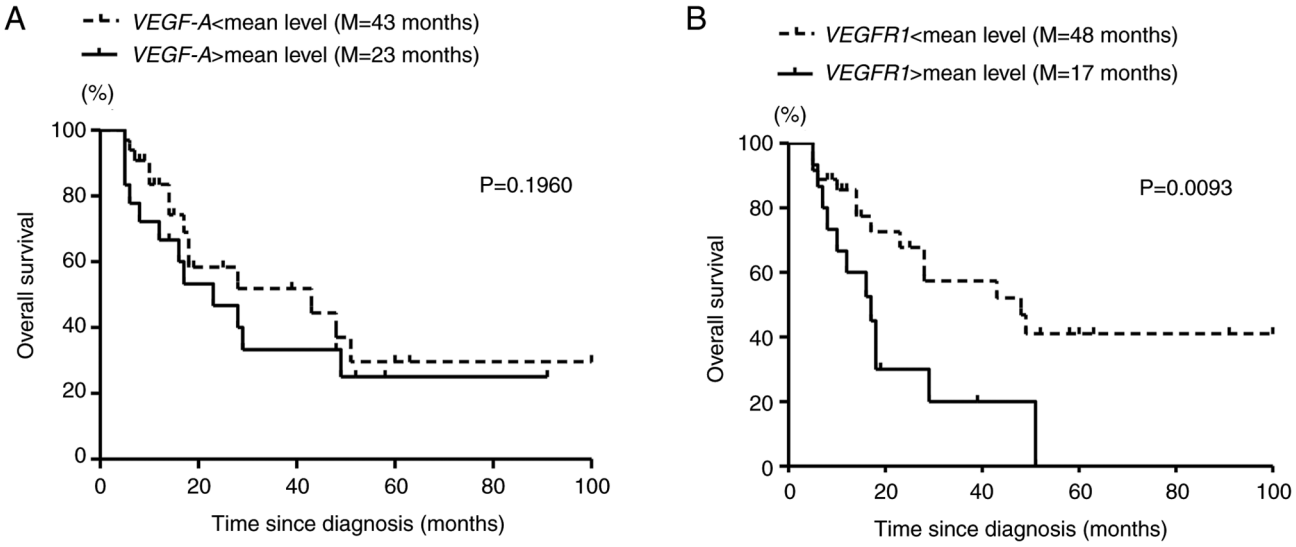

Figure 4. Kaplan-Meier survival curves of patients with myelodysplastic syndrome with high and low expression levels of (A) *VEGF-A* (mean, $1.96x10^{-2}$) and (B) *VEGFR1* (mean, $2.69x10^{-3}$). M, median.

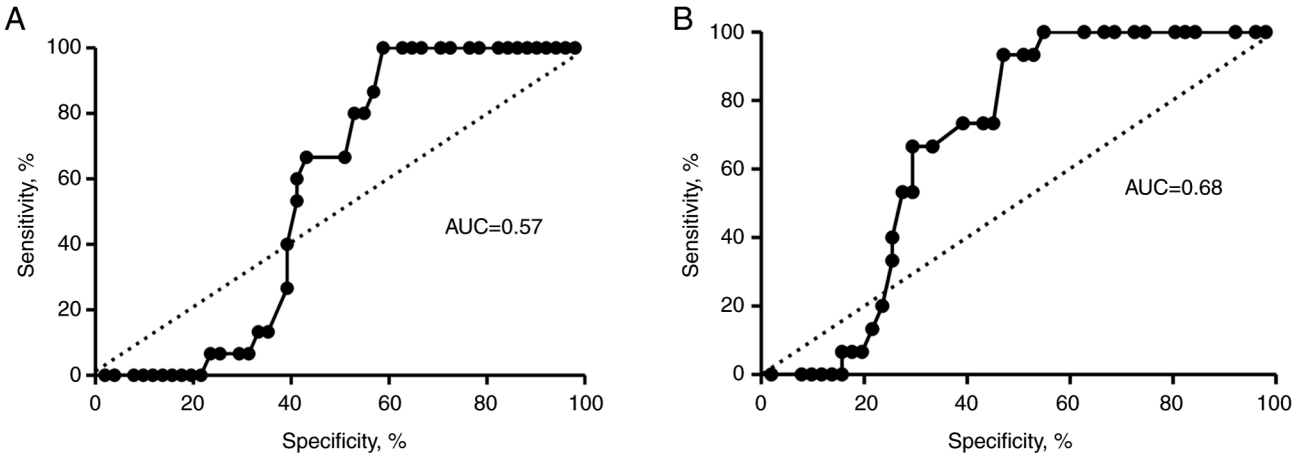

Figure 5. Receiver operating characteristic curve analysis of (A) *VEGF-A* and (B) *VEGFR1* gene expression levels for predicting diagnostic significance in patients with myelodysplastic syndrome (n=51). AUC, area under the curve.

or IPSS and final diagnosis, and found that 12% of diagnoses (109/915 patients) were reclassified. Most of the reclassified patients (67% classified according to FAB and 77% classified by IPSS) had higher risk disease. According to the WHO 2017 classification, a correct diagnosis of MDS requires the integration of not only morphological but also clinical, cytogenetic and, potentially, molecular biology parameters, thus providing an improved classification of MDS diagnosis (19). Diagnosis is more obvious in patients with excess BM blasts: Increased blast percentages (>5-10%) indicate an increase in risk of leukemic transformation and of death (28). The patients with early low-risk disease need additional diagnostic tools, including cytogenetic evaluation, flow cytometry and DNA sequencing, to define the diagnosis and predict outcomes (29). New biological markers may also be helpful to stratify patients with MDS.

VEGF and one of its receptors, VEGFR2, are prognostic factors for a number of solid tumors such as primary (30) and non-small cell lung cancer (31), neuroblastoma (32) and bladder (33), colon (34) and breast cancer (35). To the best of our knowledge, however, the data on VEGF-dependent signaling and its role in MDS development are scarce. A study showed an increase in BM microvessel density (MVD) in refractory anemia with excess blasts in transformation (RAEB-T), chronic myelomonocytic leukemia and AML compared with patients with RA, RA with ring sideroblasts and RAEB (36). *VEGF* expression in MDS specimens is especially high in RAEB ± T and AML samples (14). *VEGFR1* and, to a lesser extent, *VEGFR2* are expressed in patients with MDS and exhibit autocrine cytokine interaction (14,17).

Based on expression data of VEGF and its receptors in MDS, it was suggested that VEGF-dependent signaling could be a potential therapeutic target (7,17). A number of agents interfering with VEGF signaling, such as bevacizumab (37), SU5416 (38), AG-013736 (39), Vatalanib (40), Aflibercept/NSC 724770 (41), have been tested in patients with MDS, but yielded no or small clinical responses and clinical applicability is limited by toxicity and side effects.

The results of investigations on the VEGF prognostic impact in patients with MDS are also not encouraging. The prognostic significance of VEGF plasma levels in patients with AML and MDS has been studied (16): VEGF plasma levels are associated with a shorter survival in patients with AML, but not MDS. By contrast, elevated BM cellular VEGF levels are significantly associated with shorter survival in patients with MDS (17). Cheng *et al* (42) studied the potential prognostic value of *VEGF-A, VEGF-C,* angiopoietin-1 (*Ang-1*), *Ang-2* and receptor *Tie-2* expression in the BM of patients with MDS. *Ang-1*, but not *Ang-2*, *VEGFs* or *Tie-2*, was shown to be an independent poor prognostic factor for patients with MDS.

Despite elevated BM MVD in patients with MDS, the treatment with anti-VEGF or its receptor agents does not produce any appreciable therapeutic effects. The attempts to evaluate the potential of VEGF or its receptors to be prognostic factors for patients with MDS also were not fruitful.

In the present study, relative mRNA expression levels of VEGF (*VEGF-A*, *VEGF-C* and *VEGF-D*) and VEGF receptors (*VEGFR1*, *VEGFR2* and *VEGFR3*) in peripheral blood samples of patients with MDS were augmented compared with healthy control samples, suggesting that VEGF-dependent signaling was activated in patients with MDS. Although *VEGF-A* is activated under hypoxia, no association between *VEGF-A* expression and hemoglobin content in peripheral blood samples was found. The comparison of *VEGF* and *VEGFR* gene expression levels in patients with MDS subdivided according to IPSS risk revealed increased *VEGF-A*, *VEGFR1* and *VEGFR2* expression in higher risk groups, with the most significant difference for *VEGFR1* expression.

A key predictor of MDS development is levels of BM blast cells in patients (28) Patients with MDS with BM blast levels <5% included all patients from the IPSS system low and intermediate-1-risk groups; patients with MDS with BM blast levels >5% comprised the intermediate-2 and high-risk groups.

The difference in overall survival of patients stratified by BM blast levels (patients with >5% vs. patients with <5% blasts) was not statistically significant, but median survival time varied considerably (51 vs. 28 months, respectively). *VEGF-A*, *VEGFR1* and *VEGFR2* gene expression was upregulated in patients with MDS with BM blast levels >5% compared with patients with BM blast levels <5%. By contrast, *VEGF-C*, *VEGF-D* and *VEGFR3* gene expression levels were elevated in patients with MDS with BM blast levels <5%. This suggested that VEGF-A/VEGFR1 and VEGFR2 signaling was activated and VEGF-C and VEGF-D/VEGFR3 signaling was suppressed in patients with BM blast levels >5%.

In the present study, according to the survival curve of patients with MDS with BM blast levels <5%, two groups of patients were discriminated. The survival curve of a subgroup of these patients (group 1) largely coincided with the survival curve of patients with BM blast levels >5%, while the survival curve of patients with BM blast levels <5% (group 2) differed. The comparison of *VEGF* and *VEGFR* gene expression levels in groups 1 and 2 revealed similar gene expression, as in groups of patients with BM blast levels >5 and <5%, with the exception of *VEGF-D* and *VEGFR3* genes. As such, the elevation of certain *VEGF* (*VEGF-A*) and *VEGFR* (*VEGFR1* and *VEGFR2*) gene expression levels in patients with MDS with BM blast levels <5% may indicate the intensification of disease.

As the most prominent difference in gene expression levels between groups concerned *VEGF-A* and *VEGFR1*, the survival of patients with MDS subdivided by these gene expression levels was analyzed. A significant difference in survival was found for subgroups by *VEGFR1* expression. The survival rate of patients with MDS with *VEGFR1* expression below the mean, but not the median level of expression, was higher than in patients with higher levels of this gene expression. The difference in survival of patients subdivided by *VEGF-A* expression was not significant, but the median survival times in groups with higher *VEGF-A* expression differed significantly from those in groups with lower expression (23 vs. 43 months in groups subdivided by the mean expression and 17 vs. 48 months in groups subdivided by the median level of expression).

VEGF-A exerts its function through binding with its two specific receptors-VEGFR1 and VEGFR2, where VEGFR2 is the primary mediator of such VEGF-A biological functions as embryogenesis and hematopoiesis (43,44). According to the present study, expression of *VEGFR2*, the known negative prognostic factor for many solid tumors, (45-47) is not important in MDS. It was hypothesized that VEGF-A-dependent signaling may be preferentially realized through another receptor for VEGF-A, VEGFR1, as *VEGFR1* expression is higher in the peripheral blood samples of patients with MDS compared with *VEGFR2* expression. Previous data on *VEGF* and *VEGFR* expression levels in BM samples of patients with MDS have shown increased expression of *VEGFR1*, but not *VEGFR2* (48). Only *VEGFR1* expression in the present study had prognostic impact for patients with MDS. The elevated *VEGF-A*/*VEGFR1* expression in patients with MDS with BM blast levels >5% and patients with BM blast levels <5% with worse survival (group 1) indicated that the progression of the disease was accompanied by *VEGF-A*/*VEGFR1* activation. ROC analysis showed that *VEGFR1* expression rather than *VEGF-A* expression could serve as a potential prognostic marker in MDS with low and intermediate-1 risk.

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Availability of data and materials

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NKa, GD, NKo, AS and AK contributed to study conception and design. Clinical material preparation and data collection were performed by GD. RT-qPCR analysis was performed by NKa, NKo and AS. AK and NKa analyzed gene expression data and wrote the manuscript. GD, NKa, NKo, AS and AK confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. 6/2016) by the ethics commission of the A.S. Loginov Moscow Clinical Scientific Center (Moscow, Russia). All patients and volunteers provided written informed consent to participate in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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