VEGF Expression Correlates With Microvessel Density in Philadelphia Chromosome–Negative Chronic Myeloproliferative Disorders

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Abstract

We examined microvessel density (MVD) and immunohistochemical expression of vascular endothelial growth factor (VEGF) in the bone marrow biopsy specimens of 98 patients with Philadelphia chromosome–negative (Ph–) chronic myeloproliferative disorders (CMPDs).

*There were significantly more MVD "hot spots" in chronic idiopathic myelofibrosis (CIMF; mean ± SD, 25.6 ± 6.3) and polycythemia vera (PV; 20.7 ± 10.2) cases than in essential thrombocythemia (ET) cases (10.1 ± 4.5) and normal control (NC) samples (7.5 ± 3.6) (*P < .05*). Similar results were found using a semiquantitative method (*P < .0001*). A calculated VEGF index ($VEGF_{(i)}$) was higher in CIMF (0.29 ± 0.15) and PV (0.28 ± 0.20) cases than in ET (0.12 ± 0.05) and NC (0.08 ± 0.04) cases (*P < .0001*). MVD and $VEGF_{(i)}$ were higher in the myelofibrotic phases of CIMF and PV. There was a direct correlation between $VEGF_{(i)}$ and MVD when considering the Ph– CMPDs together (*r = 0.67; P < .001*) and when considering PV (*r = 0.79; P < .001*) and CIMF (*r = 0.40; P = .013*) as individual entities.*

Our data could provide a rationale for directly targeting VEGF or endothelial cells in CIMF and PV.

Angiogenesis is the multistep process of new capillary formation from preexisting blood vessels and has an important role in favoring the growth, dissemination, and metastatic capacity of solid tumors.[1] It has also been shown that it may be a crucial pathophysiologic mechanism in the development of hematologic malignancies. Increased microvessel density (MVD), which was first described in breast carcinoma,[2,3] has been demonstrated in many hematologic disorders, such as acute and chronic leukemia, myelodysplastic syndrome (MDS), systemic mastocytosis, and multiple myeloma.[4-6]

The few studies that have investigated bone marrow angiogenesis in Philadelphia chromosome–negative (Ph–) chronic myeloproliferative disorders (CMPDs) have reported increased MVD in myelofibrosis with myeloid metaplasia (MMM).[7-9] It has also been reported that MVD is more pronounced in chronic idiopathic myelofibrosis (CIMF) than in essential thrombocythemia (ET)[10] and that it represents an adverse predictor of survival in CIMF.[11]

It is widely accepted that the induction of angiogenesis and consequent tumor progression depends on the balance between proangiogenic and antiangiogenic factors. The most important of the multitude of proangiogenic factors is vascular endothelial growth factor (VEGF), which is capable of stimulating mitogenic activity and the proliferation of endothelial cells, increasing vascular permeability, and inducing vasodilation.[12]

High VEGF levels have been reported in a variety of hematologic malignancies, and their clinical significance has been directly related to increased MVD in acute myeloid leukemia, MDS, and multiple myeloma.[5,13-15] However, the data regarding VEGF in CMPDs are contradictory: Lundberg et al[7] found an increased number of VEGF+ cells

correlating with MVD in MMM, whereas 2 more recent studies found no difference in VEGF expression between MMM and normal control (NC) cases,[16,17] and Ni et al[18] reported increased VEGF levels in the megakaryocytes in CIMF cases.

We examined MVD and the immunohistochemical expression of VEGF in bone marrow biopsy (BMB) specimens from a large series of patients with Ph– CMPDs diagnosed on the basis of the World Health Organization classification[19] and looked for correlations between the 2 angiogenic parameters.

Materials and Methods

Cases

The study population included 98 consecutive patients (60 men and 38 women; M/F ratio, 1.6/1; median age, 61 years; range, 18-85 years) seen between January 1994 and December 2005 and followed up for a median of 21 months (range, 6-134 months). All patients gave informed consent. On the basis of clinical data, BMB specimens, and peripheral blood smears, 29 patients were given a diagnosis of ET, 30 of polycythemia vera (PV), and 39 of CIMF, according to the World Health Organization classification.[19]

In agreement with the European consensus parameters on grading bone marrow fibrosis,[20] 11 CIMF cases were graded as CIMF-0, 11 as CIMF-1, 7 as CIMF-2, and 10 as CIMF-3. Among the PV cases, 20 were classified as being in the polycythemic phase (pp-PV) and 10 in the postpolycythemic myelofibrotic phase (post-PV MF).

The NC samples consisted of 20 BMB specimens obtained for staging purposes, which were determined to be free of neoplasia and other abnormalities by histologic and immunohistochemical examinations. They were selected only from patients in the fifth and sixth decades of life whose clinical and hematologic data were within the normal range.

Clinical data at diagnosis were available for all patients and are summarized in **Table 1**.

Table 1
Clinical Data at Diagnosis for 98 Patients With Philadelphia Chromosome–Negative Chronic Myeloproliferative Disorder*

		CIMF Grade					
	ET (n = 29)	0 (n = 11)	1 (n = 11)	2 (n = 7)	3 (n = 10)	pp-PV (n = 20)	Post-PV MF (n = 10)
Age (y)	46 (18-82)	59 (27-75)	55 (29-73)	71 (61-78)	69 (60-85)	63 (38-83)	64 (45-85)
M/F (ratio)	17/12 (1.4)	6/5 (1.2)	8/3 (2.7)	5/2 (2.5)	8/2 (4)	9/11 (0.8)	6/4 (1.5)
Hemoglobin (g/dL)							
Males	14.2 (11.7-16.3)	13.8 (12.1-15.8)	12.5 (10.8-15.1)	10.8 (7.8-11.7)	10.3 (7.6-13.8)	19.2 (18.5-20.9)	11.9 (8.8-15.3)
Females	13.3 (11.3-14.8)	13.8 (12.5-14.5)	10.6 (7.6-13.3)	10.7 (9.6-11.8)	13 (11.8-14.1)	17.2 (16.5-20.1)	12.2 (9.8-14)
RBC count ($\times 10^{12}$/L)							
Males	4.6 (3.28-5.44)	4.6 (3.53-5.25)	4.3 (3.11-5.39)	3.5 (2.5-4.28)	3.3 (2.46-4.5)	7.5 (5.8-9.9)	4.4 (2.58-6.96)
Females	4.5 (3.61-5.27)	4.8 (4.28-5.05)	3.4 (2.39-4.39)	3.6 (3.37-3.89)	4.5 (4.13-4.84)	6.5 (5.6-7.7)	4.1 (3.09-5.19)
Hematocrit (%)							
Males	41.8 (34-48.6)	41.7 (35.1-46.9)	37.3 (31.3-42.9)	30.1 (22.8-35.7)	30.7 (21.3-40.7)	56 (51.6-59.1)	36.9 (26.3-49.5)
Females	40.1 (32.7-44.1)	41.3 (38-44.8)	30.8 (20.3-39.8)	31.7 (28.9-34.5)	38.3 (34.5-42.1)	52.4 (49.8-59.7)	37.6 (30.4-43.9)
MCV (fL)							
Males	91.8 (81.9-117.4)	89.8 (85.4-99.4)	87.9 (74-100.6)	86.3 (82.6-91.1)	93.2 (81.1-107)	82.8 (69.8-93.6)	87.9 (67-102.1)
Females	89.9 (72-104)	86.2 (83.3-89.5)	88.9 (85.1-91.7)	87.3 (85.8-88.8)	85.1 (83.4-86.9)	81.1 (70.5-89.7)	93.6 (73-106)
Platelet count ($\times 10^9$/L)	771 (602-1,404)	727 (289-1,099)	602 (112-1,170)	409 (105-890)	201 (17-489)	566 (132-1,028)	323 (84-903)
WBC count ($\times 10^9$/L)	7.4 (4.2-11.1)	9.9 (6.4-22)	10.4 (4.3-19.5)	10.7 (1.9-25.2)	14.7 (1.9-39.4)	10.2 (5.4-17)	10.4 (7-19.8)
CD34+ circulating cells (absolute number, mmc)	—	3.48 (1.14-23.52)	14 (0.03-114.4)	38.5 (0-101.6)	152 (19.6-439.6)	—	76.8 (33.6-273)
LDH (U/L)	391 (270-529)	471 (324-877)	899 (282-2,150)	777 (386-1,080)	1,620 (548-3,426)	481 (271-729)	926 (416-1,774)
Spleen tip (cm)†	0.6 (0-3)	0.8 (0-3)	2.6 (0-6)	6.3 (2-11)	8.2 (5-11)	1.1 (0-6)	8.6 (0-23)
Liver tip (cm)†	0.4 (0-3)	2 (0-9)	1.1 (0-2)	3.3 (1-5)	4.5 (2-9)	1.2 (0-4)	3.4 (0-7)
Bone marrow							
Aspirate blast count (%)	1.49 (0.25-4)	0.89 (0-1.75)	0.96 (0.50-2)	0.67 (0.25-1.25)	1.60 (0-5.25)	3 (0-3)	0.89 (0.25-1.50)
Cellularity (%)	45 (35-60)	55 (40-80)	66 (35-90)	65 (35-90)	45 (15-80)	75 (40-85)	70 (10-80)

CIMF, chronic idiopathic myelofibrosis; ET, essential thrombocythemia; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; pp-PV, polycythemic phase of PV; PV, polycythemia vera; post-PV MF, postpolycythemic myelofibrotic phase.

* Data are given as mean (range). Values for hemoglobin, hematocrit, and LDH are given in conventional units; conversions to Système International (SI) units are as follows: hemoglobin (g/L), multiply by 10.0; hematocrit (proportion of 1.0), multiply by 0.01; and LDH (μkat [microkatal]/L), multiply by 0.0167. Values for the RBC, platelet, and WBC counts and MCV are given in SI units; conversions to conventional units are as follows: RBC count ($\times 10^6$/μL), divide by 1.0; platelet count, ($\times 10^3$/μL), divide by 1.0; WBC count (/μL), divide by 0.001; and MCV (μm^3), divide by 1.0.

† Normal-sized spleen or liver.

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Methods

Formalin-fixed, paraffin-embedded BMB specimens obtained from the posterior superior iliac spine were available for all patients.

The BMB specimens were decalcified using an EDTA-based solution (33.27 g of EDTA and 10 mL of hydrochloric acid diluted in 1 L of distilled water) for 4 hours. Sections from each block were stained with H&E, Giemsa, Gomori silver impregnation, and Masson trichrome. Immunohistochemical analysis was performed using the automated Genomix i-6000 staining system (BioGenex, San Ramon, CA) with CD34 (clone QB-END/10, dilution 1:100; DAKO, Glostrup, Denmark), VEGF (rabbit polyclonal antibody, dilution 1:400; Santa Cruz Biotechnology, Santa Cruz, CA), myeloperoxidase (rabbit polyclonal antibody, dilution 1:3,000; DAKO), and CD61 (clone Y2/51, dilution 1.30; DAKO). Heat-induced antigen was retrieved using a 0.01 mol/L citrate solution, pH 6.0, in a microwave oven at 750 W (2 cycles for 5 minutes each). The reaction was revealed by means of the DAKO ChemMate EnVision Detection Kit (DAKO) in accordance with the manufacturer's instructions. The negative control slides were incubated with normal goat serum.

Bone Marrow MVD and VEGF Evaluation

Immunohistochemical expression for MVD and VEGF in bone marrow was evaluated in each biopsy specimen by 2 experienced pathologists (U.G. and P.R.R.), who were blinded to the clinical data and diagnosis, using a double-headed microscope.

MVD was evaluated by means of CD34 immunostaining using 2 methods.[21] The first was the so-called hot spots method (MVD-HS),[22] in which each BMB specimen was first totally scanned at low magnification (10×) to identify 3 hot spot zones. MVD-HS was then estimated at high magnification (40×) by counting all positively stained endothelial cells or endothelial cell aggregates that were clearly separated from adjacent microvessels. Sinusoid-like structures (but not arterioles) were also counted. Megakaryocyte precursors and clusters of myeloid precursors expressing the CD34 antigen (respectively identified on the basis of their different morphologic features and CD61 and myeloperoxidase expression) were excluded from the evaluation. The second was a semiquantitative method (MVD-SQ)[8] based on the following scale: grade 1, the vascularity in an NC sample; grade 2, slightly increased MVD in comparison with an NC sample; grade 3, moderately increased MVD in comparison with an NC sample; and grade 4, markedly increased MVD in comparison with an NC sample.

To avoid bias related to variations in the cellularity of BMB specimens in CMPD cases, we calculated a VEGF expression index ($VEGF_{(i)}$), defined as the cellularity of the BMB specimen multiplied by the fraction of VEGF+ cells and expressed as a number between 0 and 1 [(% of BMB Specimen Cellularity × % of VEGF+ Cells)/10^4].[23]

JAK2 Mutational Status

Genomic DNA was extracted from peripheral whole blood or purified granulocytes using standard manual methods.[24] The $JAK2^{V617F}$ mutation was detected by means of allele-specific polymerase chain reaction according to the protocol of Baxter et al.[25] Mutational status was confirmed by direct sequencing (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, Warrington, England) using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

Statistical Analysis

The data were statistically evaluated using the SPSS 11 statistical package (SPSS, Chicago, IL). The hypothesis tests were 2-sided, and the other statistical tests were performed at the 5% significance level ($P < .05$). The demographic and anamnestic data were analyzed descriptively (frequencies and percentages for qualitative variables; means, medians, and SD [± SD] for quantitative variables).

The homogeneity of the population was verified by using the χ^2 test for qualitative parameters and 1-way analysis of variance (ANOVA) for quantitative data. The MVD and VEGF expression findings in the Ph– CMPDs and the subgroups of each Ph– CMPD were evaluated by means of the multiple range test (Bonferroni modified test).

Results

MVD and VEGF Index in Ph– CMPDs

The MVD-HS and $VEGF_{(i)}$ results are summarized in **Table 2**. The MVD-HS method revealed statistically significant differences between NC (7.5 ± 3.6), ET (10.1 ± 4.5), PV

Table 2
Microvessel Density "Hot Spots" and VEGF Index in 98 Philadelphia Chromosome–Negative Cases of Chronic Myeloproliferative Disorders and 20 Control Cases*

	Microvessel Density Hot Spots	VEGF Index
Normal control cases	7.5 ± 3.6	0.08 ± 0.04
Essential thrombocythemia	10.1 ± 4.5	0.12 ± 0.05
Chronic idiopathic myelofibrosis	25.6 ± 6.3	0.29 ± 0.15
Chronic phase	24.8 ± 6.5	0.25 ± 0.15
Myelofibrotic phase	26.0 ± 6.3	0.32 ± 0.16
Grade		
0	24.8 ± 6.5	0.25 ± 0.15
1	26.1 ± 6.4	0.33 ± 0.17
2	25.3 ± 4.9	0.37 ± 0.17
3	26.3 ± 7.6	0.27 ± 0.14
PV	20.7 ± 10.2	0.28 ± 0.20
pp-PV	15.2 ± 5.9	0.19 ± 0.11
Post-PV MF	31.9 ± 7.4	0.49 ± 0.2

pp-PV, polycythemic phase of PV; PV, polycythemia vera; post-PV MF, postpolycythemic myelofibrotic phase; VEGF, vascular endothelial growth factor.
* Data are given as mean ± SD.

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(20.7 ± 10.2), and CIMF (25.6 ± 6.3) cases **Figure 1A** ($P < .0001$; ANOVA), being significantly greater in PV and CIMF cases than in ET and NC cases ($P < .05$; multiple range test analysis, Bonferroni LSD) **Image 1**. The MVD-SQ results were similar ($P < .0001$; Kruskal-Wallis 1-way ANOVA).

A significant difference was also found when the chronic and myelofibrotic phases of PV and CIMF were evaluated separately **Figure 1B** ($P < .0001$; ANOVA), with higher MVD-HS levels found in the fibrotic phases of the diseases and a statistically significant difference when comparing pp-PV (15.2 ± 5.9) and post-PV MF (31.9 ± 7.4) ($P < .05$; multiple range test analysis, Bonferroni LSD). These findings were confirmed by MVD-SQ ($P < .0001$; Kruskal-Wallis 1-way ANOVA; and $P < .05$; multiple range test analysis, Bonferroni LSD). Statistical analysis of CIMF cases did not reveal significant differences between the different histologic grades.

For VEGF, we first examined protein expression in normal bone marrow cells and found moderately to markedly intense VEGF immunoreactivity in the myeloid lineage at all stages of maturation, in megakaryocytes (variable expression), in histiocytes, and in plasma cells. The $VEGF_{(i)}$ revealed different levels of protein expression between NC (0.08 ± 0.04), ET (0.12 ± 0.05), CIMF (0.29 ± 0.15), and PV (0.28 ± 0.20) cases **Figure 1C** ($P < .0001$; ANOVA), with higher levels in CIMF and PV cases than in ET and NC cases ($P < .05$; multiple range test analysis, Bonferroni LSD) **Image 2**.

$VEGF_{(i)}$ demonstrated higher levels of protein expression in the fibrotic phases of CIMF and PV, particularly in PV (pp-PV, 0.19 ± 0.11 vs post-PV MF, 0.49 ± 0.2) ($P < .05$;

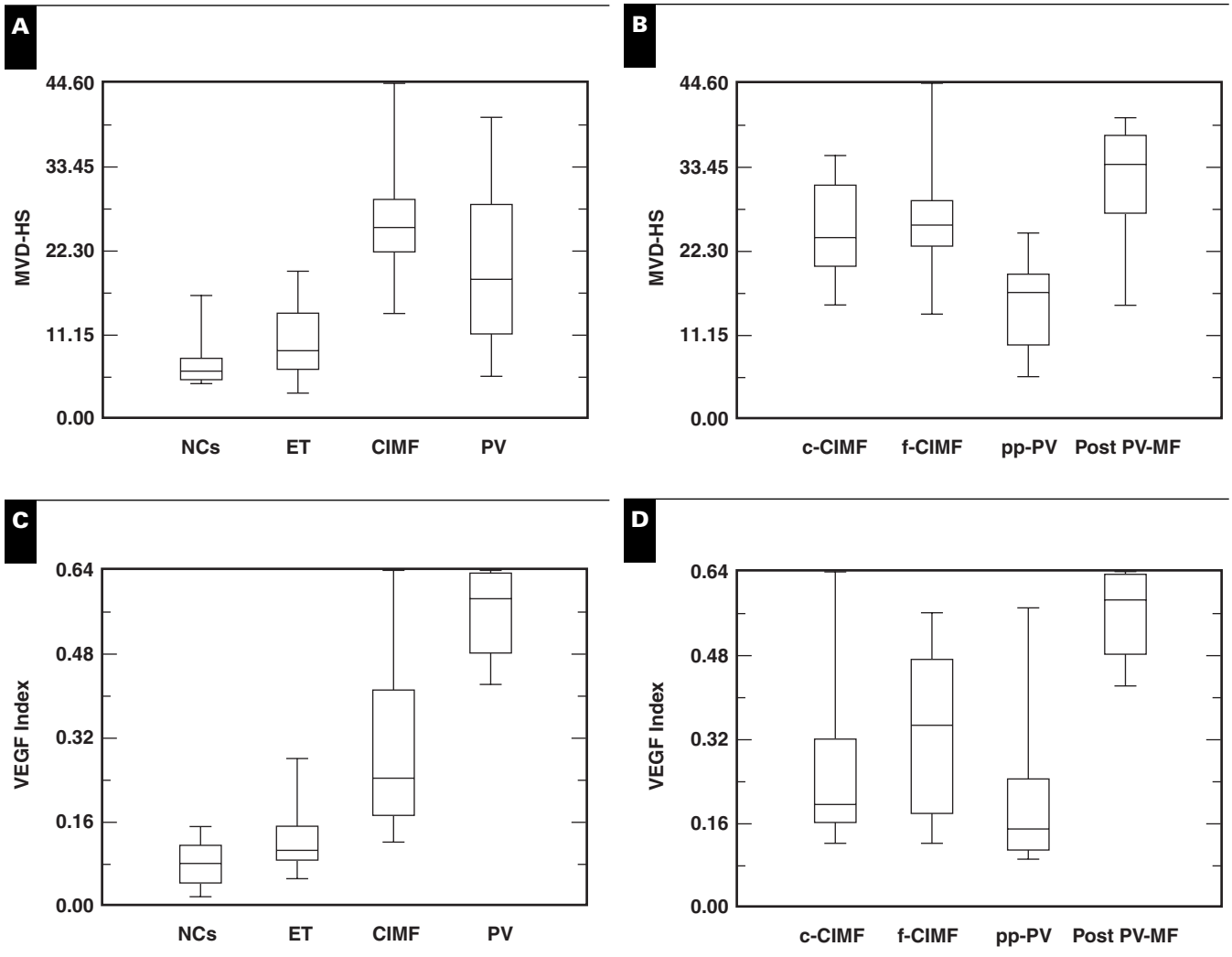

Figure 1 **A** and **B**, Microvessel density "hot spots" (MVD-HS) in cases of Philadelphia chromosome–negative chronic myeloproliferative disorders (**A**; Ph– CMPDs) and the chronic (c-CIMF) and myelofibrotic (f-CIMF) phases of chronic idiopathic myelofibrosis (CIMF) and the polycythemic (pp-PV) and postpolycythemic myelofibrosis (post-PV MF) phases of polycythemia vera (**B**; PV). **C** and **D**, Vascular endothelial growth factor (VEGF) index in cases of Ph– CMPD (**C**) and c-CIMF, f-CIMF, pp-PV, and post-PV MF (**D**). The box-whisker plots indicate the minimum value, first quartile, mean value, third quartile, and maximum value.

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multiple range test analysis, Bonferroni LSD) **Figure 1D**. There were no significant differences in VEGF levels between the grades of CIMF.

Correlation Between VEGF Index and MVD-HS in Ph– CMPDs

A direct correlation was found between the $VEGF_{(i)}$ and MVD-HS in the BMB specimens of Ph– CMPD cases considered as a whole ($r = 0.67$; $P < .001$) **Figure 2**. Furthermore, there was a statistically significant correlation in the BMB specimens of PV cases ($r = 0.79$; $P < .001$) **Figure 3** and CIMF cases ($r = 0.40$; $P = .013$) considered as individual entities.

JAK2 and MVD in Ph– CMPDs

JAK2 mutational status was studied in a subset of 38 cases. The $JAK2^{V617F}$ mutation was found in 9 (56%) of 16 cases of CIMF (3 homozygous and 6 heterozygous), 13 (87%) of 15 cases of PV (5 homozygous and 8 heterozygous), and 4 (57%) of 7 cases of ET (1 homozygous and 3 heterozygous). We found no significant correlation between JAK2 mutational status and MVD or $VEGF_{(i)}$.

Discussion

In this study, we examined MVD and immunohistochemical expression of VEGF in BMB specimens obtained from patients

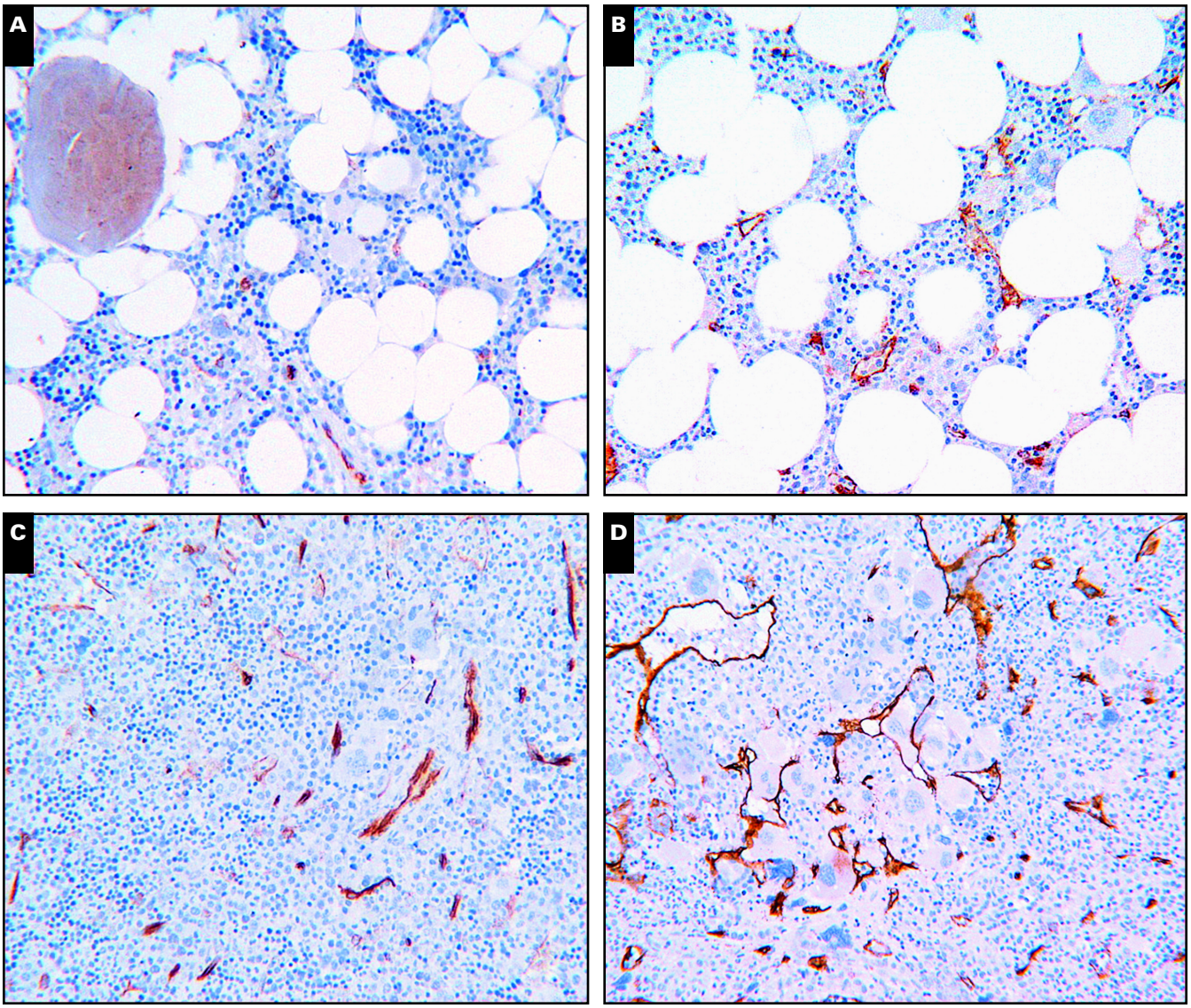

Image 1 Microvessel density (MVD) in Philadelphia chromosome–negative chronic myeloproliferative disorders evaluated using CD34 antibody. MVD was not significantly different between the normal control samples (**A**) and essential thrombocythemia (**B**) but was greater in polycythemia vera (**C**) and chronic idiopathic myelofibrosis (**D**) (**A**-**D**, CD34 immunostaining, diaminobenzidine chromogen, ×10).

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with Ph– CMPDs. In comparison with NC and ET cases, PV and CIMF cases showed significantly higher MVD as evaluated by the MVD-HS and MVD-SQ methods. Interestingly, we found no significant difference in MVD between the NC and ET cases.

MVD was significantly higher in myelofibrotic than in chronic phases of CIMF and PV, a difference that was statistically significant in PV cases. In contrast, there were no significant differences between the CIMF cases with different grades of bone marrow fibrosis.

Our MVD data are in line with those of previous studies describing increased angiogenesis in CMPDs.[8-11,18,21] Interestingly, we found the highest levels of MVD in PV, particularly in post-PV MF.

The published data regarding proangiogenic VEGF expression in CMPDs are controversial. Increased serum levels have been documented in idiopathic myelofibrosis,[15] and some positive immunohistochemical evidence has been reported. An increased number of VEGF+ cells in MMM correlates with MVD,[7] higher VEGF levels have been found in the bone marrow of patients with myeloproliferative disorders,[26] and there is a recent description of strong immunohistochemical VEGF expression in the megakaryocytes of idiopathic myelofibrosis.[18] In contrast, 2 recent studies found no differences in the distribution and intensity of VEGF immunostaining between MMM and control cases.[16,17]

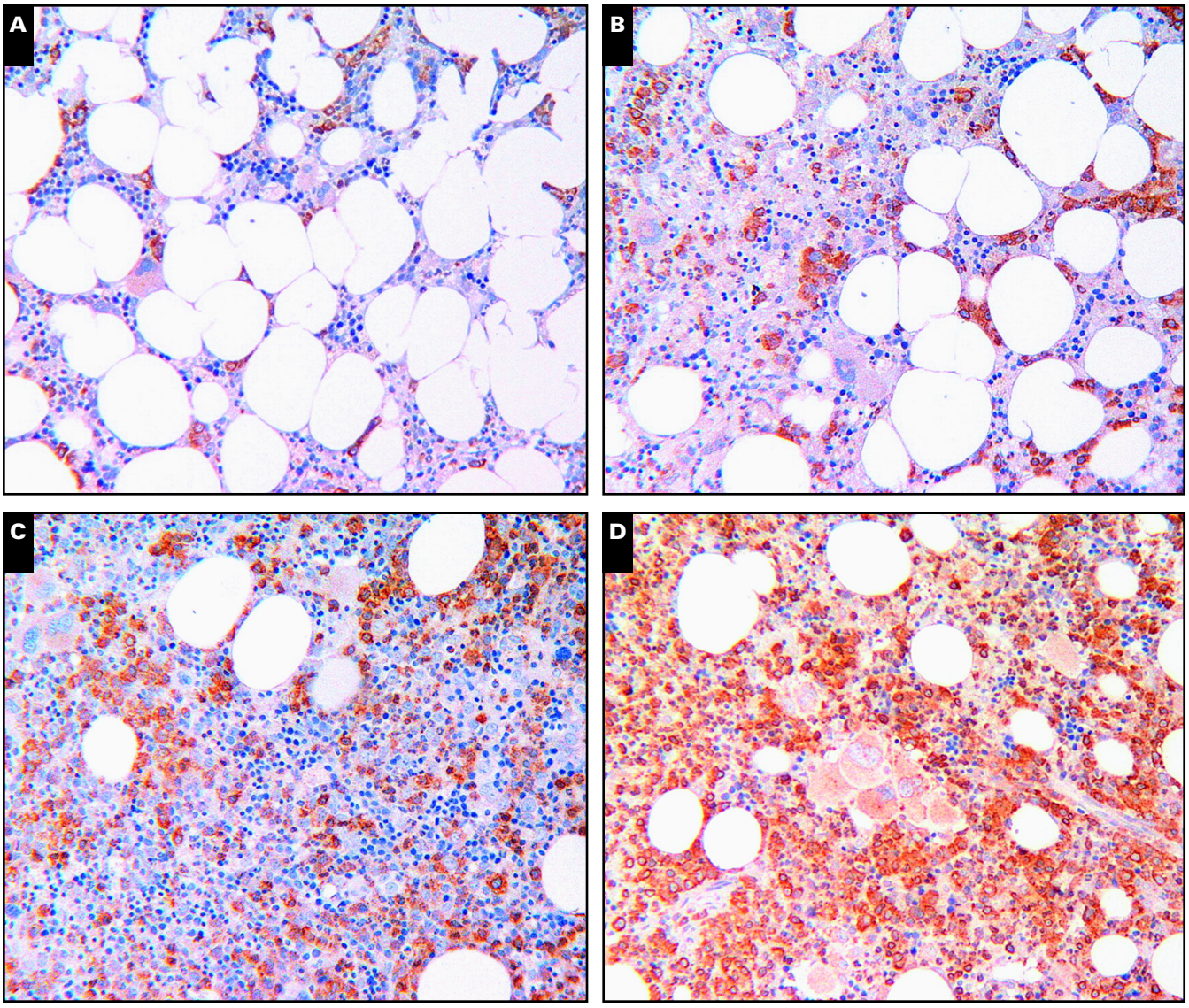

Image 2 Vascular endothelial growth factor (VEGF) immunohistochemical expression in Philadelphia chromosome–negative chronic myeloproliferative disorders. VEGF expression was lower in the normal control samples (**A**) and essential thrombocythemia (**B**) but higher in polycythemia vera (**C**) and chronic idiopathic myelofibrosis (**D**) (**A**-**D**, VEGF immunostaining, diaminobenzidine chromogen, ×10).

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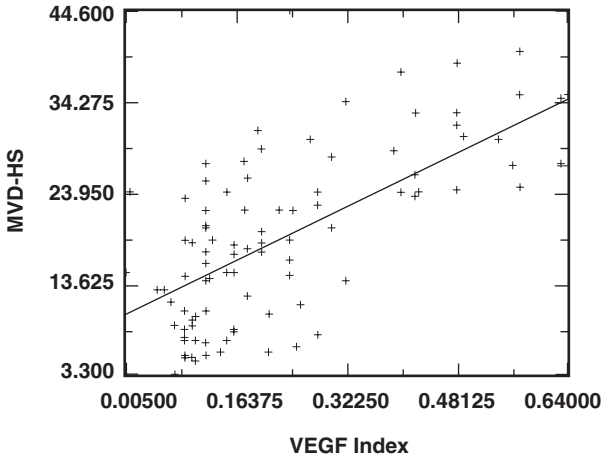

Figure 2 Linear correlation between the vascular endothelial growth factor (VEGF) index and microvessel density "hot spots" (MVD-HS) in 98 cases of Philadelphia chromosome–negative chronic myeloproliferative disorders. $r = 0.67555$; $P < .001$.

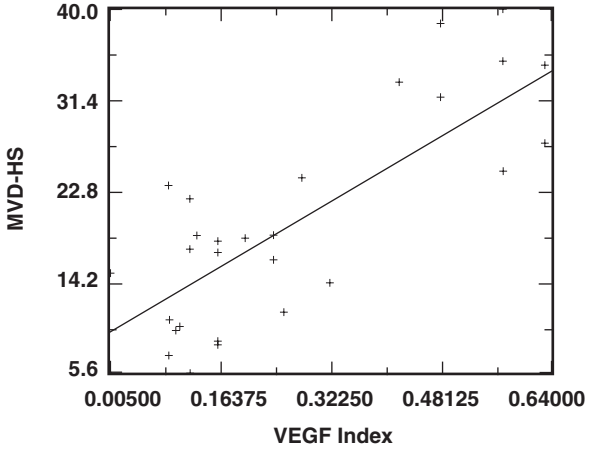

Figure 3 Linear correlation between the vascular endothelial growth factor (VEGF) index and microvessel density "hot spots" (MVD-HS) in 30 cases of polycythemia vera. $r = 0.79235$; $P < .001$.

We found that the immunohistochemical expression of VEGF parallels the increase in MVD. The $VEGF_{(i)}$ was significantly higher in CIMF and PV cases than in ET and NC cases. It was also higher in the myelofibrotic phases of CIMF and PV, but the difference was significant only in PV. There was no difference between cases with different degrees of bone marrow fibrosis.

Our findings are in line with those of Lundberg et al[7] and Wrobel et al[26] but not with those of Chou et al[16] or Ho et al.[17] There may be various reasons for the discrepancies, the most important of which are likely to be the use of different CMPD classification schemes and the different method of evaluating immunohistochemical VEGF expression. Regarding the latter question, we decided to use a $VEGF_{(i)}$ to avoid bias related to the variability in bone marrow cellularity that frequently characterizes CMPDs. The index was calculated in a manner similar to that used to calculate the hairy cell index, which is used to evaluate the entity of bone marrow involvement in hairy cell leukemia.[23] In a previous study evaluating survivin expression in MDS, Gianelli et al[27] demonstrated the usefulness of this method of quantifying protein expression in BMB specimens and its correlation with messenger RNA levels.

To the best of our knowledge, this study is the first to document a direct correlation between VEGF levels and MVD in a large series of Ph– CMPD cases ($r = 0.67$; $P < .001$). This correlation was more evident in the BMB specimens of PV cases ($r = 0.79$; $P < .001$), but can also be seen in CIMF cases ($r = 0.40$; $P = .013$). A direct correlation between VEGF and MVD has been reported in other hematologic malignancies such as acute myeloid leukemia, MDS, and multiple myeloma.[5,15] In particular, it has been postulated that VEGF may act in a paracrine manner in contributing to the increased MVD in these malignant conditions, and a similar mechanism may stimulate the proliferation of new vessels in the bone marrow of patients with Ph– CMPDs.

It is well known that ET differs from other CMPDs in terms of reticulin fiber content, and we recently reported a statistically significant absence of trabecular bone remodeling in the BMB specimens of ET cases in comparison with CIMF cases.[28] Moreover, our MVD and VEGF data indicate that, also in relation to the angiogenic process, ET cases seem to be more similar to NC cases than to the other Ph– CMPD cases.

In contrast, our results show that PV and CIMF are characterized by increased angiogenesis and the increased expression of proangiogenic VEGF. These considerations may be of some relevance in the context of new therapeutic approaches aimed at directly targeting endothelial cells or VEGF. In particular, it is interesting to note that thalidomide, thalidomide analogues, and new agents acting on the angiogenic process via the VEGF pathway are under clinical investigation in MMM.[29]

Finally, we also studied JAK2 mutational status in a subset of Ph– CMPD cases. We found no significant correlations between the $JAK2^{V617F}$ mutation and MVD or $VEGF_{(i)}$ but, given the limited number of observations, further studies are needed to verify this finding.

Our study demonstrates that patients with Ph– CMPDs (particularly PV and CIMF) have increased MVD and VEGF levels. The increased MVD parallels the expression of VEGF, and there is a direct correlation between the 2 angiogenic parameters. Our data could offer a rationale for clinical trials of drugs aimed directly at target endothelial cells or VEGF in CIMF and patients with PV. Further studies of larger numbers of patients are needed to study the correlation between JAK2 mutational status and bone marrow angiogenesis.

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