

# Plasmablastic multiple myeloma is associated with increased vascular endothelial growth factor immunoexpression

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## Abstract

The biologic basis of the negative prognosis of plasmablastic myeloma is not fully understood. To determine whether histologically aggressive multiple myeloma (MM) is associated with a more angiogenic marrow environment, bone marrow samples from 50 recently diagnosed MM patients were evaluated. Twelve percent (6/50) of patients presented plasmablastic MM, and this feature correlated with moderate/strong intensity of vascular endothelial growth factor staining of plasma cells ( $P = 0.036$ ). Although plasmablastic MM was not associated with increasing of microvessel density, this new evidence of increased expression of vascular endothelial growth factor on plasmablasts suggests that the adverse prognosis conferred by plasmablastic disease may be due, at least in part, to secretion of this angiogenic cytokine, also suggesting that the subset of MM patients with plasmablastic features may derive particular benefit from antiangiogenic therapies.

## Key words

- Multiple myeloma
- Angiogenesis
- Vascular endothelial growth factor

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## Introduction

The amount of microvessels in the bone marrow progressively increases along the spectrum of plasma cell disorders and becomes a prominent feature in active multiple myeloma (MM) (1). Some studies have established that MM patients with increased marrow angiogenesis have shorter overall survival (2,3). Vascular endothelial growth factor (VEGF) is one of the most important angiogenic cytokines mediating tumor an-

giogenesis (4); assessed by immunohistochemical methods, it has been detected in plasma cells and plasmacytoma samples from most MM patients (5,6).

Based on the hypothesis that the adverse clinical evolution associated with plasmablastic MM (7,8) could be determined by an increase of angiogenesis, we studied the correlation between plasmablastic morphology, microvessel density (MVD) and VEGF immunoexpression in the bone marrow samples of 50 newly diagnosed MM patients. If this

hypothesis were correct, this subset of MM patients would be particularly suitable for antiangiogenic therapies.

## Material and Methods

### Patients

We studied bone marrow samples from fifty recently diagnosed MM patients, followed at the Multiple Myeloma Outpatient Service of the Discipline of Hematology and Hemotherapy, UNIFESP/EPM, São Paulo, SP, Brazil, between May 2000 and December 2002. Formalin-fixed, paraffin-embedded bone marrow sections from 49 patients were available, and 46 patients had also assessable bone marrow smears. Written informed consent was obtained from all patients and the institution's Ethics Committee approved the study.

### Assessment of morphologic features

Marrow smears were stained with May-Grünwald-Giemsa solution to evaluate plasma cell number and morphology. Bone marrow sections were studied after staining with hematoxylin and eosin, Giemsa and PAS. The disease was classified as plasmablastic or non-plasmablastic according to the classification system of Bartl et al. (9), and the percentage of infiltrating plasma cells (tumor burden) was recorded.

### Immunohistochemical studies

Immunohistochemical reactions were performed using the streptavidin-biotin-peroxidase method and the following monoclonal antibodies: anti-kappa light chain (1:5000; Dako, Carpinteria, CA, USA), anti-lambda light chain (1:5000; Dako), anti-CD34 (1:50; Dako), and anti-VEGF (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The LSAB detection kit (Dako) was used for antigen visualization. Paraffin sec-

tions of placental tissue were included in each batch as positive control for CD34 immunohistochemical staining and sections processed with exclusion of the primary antibody were used as negative control. Sections were counterstained with hematoxylin and examined by light microscopy.

### Quantification of microvessels in bone marrow

Angiogenesis was measured by counting the CD34-labeled intratumoral microvessels (MVD) (1), and the patients were stratified into high-angiogenesis (MVD  $\geq 20$ ) and low-angiogenesis status (MVD  $< 20$ ) according to a previous study (10). To estimate the MVD, the entire slide was scanned at 100X magnification to locate three areas of maximum microvessels (hot spots) in which the microvessels were counted at 400X. The MVD was reported as the average number of vessels in the three hot spots (1).

### Assessment of VEGF expression

Cases were considered to be positive when  $\geq 10\%$  of plasma cells showed the typical pattern of VEGF expression, evidenced by diffuse cytoplasmic brown staining of variable intensity among individual patients. For the positive cases, VEGF immunoreactivity was examined on the basis of staining intensity (weak and moderate/strong). Given the known VEGF expression by marrow myelo-monocytic and megakaryocytic cells, which invariably showed strong VEGF staining, these cells were used as positive internal controls (4,5).

### Statistical analysis

Associations between nominal variables were tested by the Pearson chi-square test ( $\chi^2$ ) or by the Fisher exact test, with the level of significance set at 5%. Statistical analysis was performed using the SPSS 8.0 software.

## Results

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

### Histological characteristics

Bone marrow biopsies were available for 49 patients. Plasma cell infiltration in marrow samples ranged from 20 to 100% (median 80%). According to the classification of Bartl et al. (9), 6 patients (12%) presented plasmablastic disease, while 43 (88%) did not (Figure 1).

### Bone marrow angiogenesis

Forty-eight CD34-stained marrow sections were available to assess angiogenesis. Median MVD was 25 microvessels per 400 high-power field (range: 3-130). Low angiogenesis (MVD <20) was present in 13 (27%) patients, and high angiogenesis (MVD  $\geq$ 20) was present in 35 patients (73%).

### VEGF expression

Forty-nine sections were assessable for VEGF analysis. Fourteen (29%) patients showed VEGF expression in <10% positive plasma cells (negative cases), while 35 (71%) showed VEGF expression in  $\geq$ 10% positive plasma cells. Among the positive cases, 17

(49%) displayed a weak intensity signal, while 18 (51%) showed moderate/strong intensity.

### Correlations between morphologic feature, VEGF expression and bone marrow angiogenesis

Plasmablastic morphology did not show an association with tumor burden in bone

Table 1. Clinical characteristics of the 50 patients studied.

Patients' baseline characteristics	N
Age (years)	
Median	57
Range	28-83
Gender	
Male	29 (58%)
Female	21 (42%)
Salmon-Durie stage	
IIa	2 (4%)
IIb	1 (2%)
IIIa	19 (38%)
IIIb	28 (56%)
Isotype	
IgG	24 (48%)
IgA	10 (20%)
IgM	1 (2%)
Not IgM, IgA, IgG	12 (24%)
Data missing	3 (6%)
Light chain	
Kappa	27 (54%)
Lambda	18 (36%)
Not available	5 (10%)

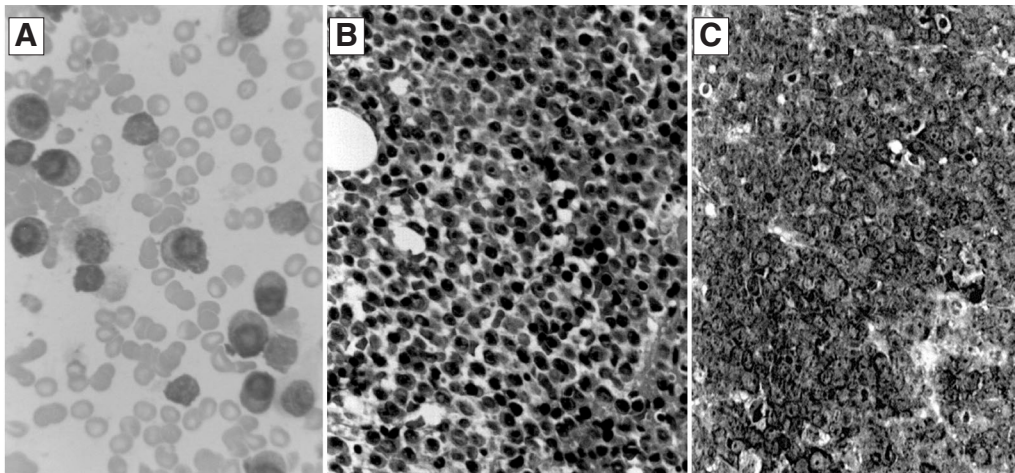


Figure 1. Micrographs showing plasmablasts in marrow smears (A), plasmablasts in a marrow biopsy (B), and plasmablasts with strong vascular endothelial growth factor immunostaining in a marrow biopsy (C). Original magnification, 400X.

marrow biopsies ( $P = 0.614$ ); also, there was no association with angiogenesis status, as assessed by MVD ( $P = 0.713$ ). However, plasmablastic MM significantly correlated with moderate/strong VEGF intensity ( $P = 0.036$ ; Table 2).

## Discussion

Plasmablastic morphology is a well-recognized adverse morphologic feature in MM (7,8). Findings from clinical and laboratory studies have suggested that angiogenesis takes part in the pathogenesis of MM (11), and some studies have revealed angiogenesis as a prognostic factor for disease-related survival and as a significant predictor of plasmacytoma progression to MM (2,3).

The goal of this study was to investigate the potential links between plasmablastic morphology and angiogenesis in a series of advanced, recently diagnosed MM patients, hypothesizing that a histologically aggressive disease would also correspond to a more angiogenic bone marrow environment. Assessing VEGF immunoexpression in marrow biopsies in terms of staining intensity, we arbitrarily set  $\geq 10\%$  of positive plasma

cells as the cut-off for considering a case as positive.

Bellamy et al. (4) studied the VEGF mRNA and protein expression in 12 human hematopoietic tumor cell lines and in 16 MM patients. VEGF expression was defined according to the intensity of cytoplasmic staining; immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded bone marrow clots or cores, and plasma cell expression of VEGF was observed in 12 of 16 patients, presenting as diffuse cytoplasmic staining of variable intensity among individual patients. With an expanded cohort of 42 MM patients, using the same immunohistochemistry method, Bellamy (5) was able to observe plasma cell expression of VEGF in the bone marrow of 33 (78%) patients.

We found an interesting association between plasmablastic morphology and moderate/strong VEGF intensity even though we did not detect a correlation between plasmablastic morphology and angiogenesis status. This finding, although limited by the small number of patients with plasmablastic features, suggests that a histologically more aggressive disease may be associated with intensified production of VEGF. Although VEGF expression has been significantly associated with immature morphology in plasmacytomas (6), the correlation in MM has not been previously reported.

Rajkumar et al. (1) analyzed angiogenesis in 400 patients with plasma cell dyscrasias regarding medullary microvessels identified by immunohistochemical staining for CD34. From the relatively benign monoclonal gammopathy of undetermined significance to relapsed MM, the median MVD increased significantly and high-grade angiogenesis found to be progressively more frequent, suggesting that angiogenesis may be related to disease progression.

The high-angiogenesis status shared by 73% of our patients probably reflects the virtually homogenous group of stage III dis-

Table 2. Correlation between the classification of Bartl et al. (9) and vascular endothelial growth factor intensity and microvessel density in multiple myeloma patients.

Bartl's classification	Plasmablastic (N)	Non-plasmablastic (N)
PC BM (%)		
<80%	2 (33%)	10 (24%)
$\geq 80\%$	4 (67%)	32 (76%)
VEGF intensity*		
Negative	0	14 (33%)
Weak	1 (17%)	16 (37%)
Moderate/strong	5 (83%)	13 (30%)
Angiogenesis status		
Low (MVD <20)	2 (33%)	11 (26%)
High (MVD $\geq 20$ )	4 (67%)	31 (74%)

PC BM = plasma cells in bone marrow (median 80); VEGF = vascular endothelial growth factor; MVD = microvessel density.

\*Plasmablastic multiple myeloma significantly correlated with moderate/strong VEGF intensity ( $P = 0.036$ )

ease, highlighting the association of angiogenesis with advanced MM. Although we did not detect an association between plasma cell percentage and MVD or VEGF, the hot spots were generally found to be closely related to focal areas of tumor infiltration (data not shown), suggesting that autocrine production of angiogenic cytokines was taking place.

In short, our findings suggest that plasmablastic MM is associated with increased production of VEGF and confirm the association of advanced MM with increased angiogenesis. In view of current efforts to study antiangiogenic drugs in the treatment of MM (12), we believe that this subset of MM patients could benefit from these therapies.

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