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Original Research Article

VEGF-A, sVEGFR-1, and sVEGFR-2 in BCR-ABL negative myeloproliferative neoplasms

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ABSTRACT

Background and objective: Data from the literature indicate the relationship between the bone marrow microvessel density and the blood parameters of angiogenesis. The aim of this study was to evaluate selected parameters of angiogenesis (VEGF-A, sVEGFR-1, and sVEGFR-2) and their correlations with white blood cells, platelets, and red blood cells.

Materials and methods: The study included 72 patients (mean age, 61.84 years) with myeloproliferative neoplasms (MPNs): essential thrombocythemia (ET) ($n = 46$), polycythemia vera (PV) ($n = 19$), and primary myelofibrosis (PMF) ($n = 7$). Serum VEGF-A, sVEGFR-1, and sVEGFR-2 were determined using the ELISA assay.

Results: We observed a significantly higher level of VEGF-A and reduced concentrations of sVEGFR-1 and sVEGFR-2 in the whole group of patients with MPNs as compared to controls. Detailed analysis confirmed significantly higher level of VEGF-A and lower concentration of sVEGFR-2 in each subgroups of MPNs patients. However, sVEGFR-1 concentrations were significantly lower only in PV and ET patients.

Conclusions: The study showed an increased level of VEGF-A, which may indicate the intensity of neoangiogenesis in the bone marrow. Decreased sVEGFR-1 and sVEGFR-2 in the blood of patients with MPNs may reflect consumption of these soluble receptors.

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1. Introduction

Myeloproliferative neoplasms (MPNs) are the result of clonal proliferation of stem cells of bone marrow, characterized by the proliferation of one or more myeloid lines (granulocyte, erythrocyte, and megakaryocyte) [1]. Classic myeloproliferative neoplasms include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).

Since 2008, a new WHO classification distinguishes BCR-ABL-negative myeloproliferative neoplasms including essential thrombocythemia, polycythemia vera and primary myelofibrosis. This group of diseases is identified on the basis of mutations in multipotent stem cells and defined as a lack of Ph chromosome (and the fusion gene BCR-ABL) and potentially allows the presence of the *JAK2* mutation.

In recent years, significant progress has been made in understanding angiogenesis process that plays a key role in the pathogenesis of myeloproliferative neoplasms. Regulation of angiogenesis in cancer is complex and depends on angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGFα), tumor necrosis factor (TNF), interleukin-6, interleukin-8, epidermal growth factor (EGF), angiopoietin-1, angiogenin, integrin and anti-angiogenic factors such as thrombospondin-1, angiostatin, endostatin, fibronectin, interferon-α, interferon-γ, interleukin-1, interleukin-12, platelet factor 4.

The most specific endothelial cell growth factor is vascular endothelial growth factor (VEGF) [2]. VEGF is a homodimeric glycoprotein of approximately 45 kDa. VEGF family includes seven proteins: VEGF-A, -B, -C, -D, -E, -F, and placental growth factor (PIGF) [3]. The best known factor is VEGF-A, whose gene is located on the short arm of chromosome 6 (6p 21.3) [4]. VEGF is produced by various cells such as macrophages, T lymphocytes, endothelial cells, vascular smooth muscle cells and tumor cells [5]. VEGF exerts its effect by binding to specific receptor VEGFR-2, located on the vascular endothelial cells. Currently there are known two soluble forms of the receptors for VEGF: sVEGFR-1 and sVEGFR-2. The soluble form of VEGFR-1 lacks the seventh immunoglobulin-like domain, transmembrane region, and intracellular signaling tyrosine kinase domain characteristic of VEGFR-1 and VEGFR-2. It is believed that sVEGFR-1, via connecting to each isoform of VEGF, acts as an inhibitor of angiogenesis, reducing VEGF availability to endothelium [6–8]. Currently, very little is known about sVEGFR-2, a product of ectodomain shedding from cell-surface VEGFR-2 or an alternative mRNA splice variation. According to recent studies, sVEGFR-2 is a natural inhibitor of angiogenesis [9].

Many studies have shown that angiogenesis plays an important role in the pathogenesis of myeloproliferative neoplasms. Due to the fact that bone marrow is the site of tumor cell formation, early studies focused on the bone marrow microvascular density assessment. An increased density of vessels has been demonstrated mostly in PMF and PV patients. Significantly higher concentration of VEGF-A in patients with myeloproliferative neoplasms correlated with microvascular density, as well as with the clinical severity of the disease progression. For this reason, VEGF-A may be used as a diagnostic and prognostic marker of MPNs [7].

The aim of this study was to measure VEGF-A, sVEGFR-1 and sVEGFR-2 concentrations in patients with myeloproliferative neoplasms and to examine their correlations with other parameters (number of leukocytes, erythrocytes and platelets).

2. Materials and methods

The study involved 72 newly diagnosed patients (mean age, 61.84 years) with myeloproliferative neoplasms, hospitalized and diagnosed at the Hematology Clinic Dr. J. Biziel University Hospital No. 2 in Bydgoszcz, Poland. The study group included 46 patients with ET, 19 with PV, and 7 with PMF. Patients at the time of inclusion in the study were not taking drugs affecting angiogenesis. The diagnosis of ET was based on the diagnostic criteria of ET according to the WHO (2008) [10] and the exclusion of other malignant and non-malignant diseases in the course of which there can be observed essential thrombocythemia. The diagnosis of PV was based on the diagnostic criteria for PV according to the WHO (2008) [10] including a genetic test for the gene *JAK2* V617F mutation. In all patients with PV there were initially excluded secondary causes of PV. Myelofibrosis diagnosis was based on the diagnostic criteria for spontaneous bone marrow fibrosis, according to the WHO (2008) [10], including the study on the presence of cytogenetic V617F mutation in gene *JAK2*.

Mutational status of MPNs patients and *JAK2* V617F-negative patients is presented in Tables 1 and 2.

The control group consisted of 39 healthy volunteers (mean age, 59.22 years) who were age and sex matched.

The material for the test was venous blood collected from the elbow vein into 2 tubes containing different anticoagulants: 3.2% sodium citrate and K2EDTA. Peripheral blood counts were performed on Advia 120 hematology analyzer. Concentration of fibrinogen was marked using the Siemens

Table 1 – Mutational status of MPNs patients.

	Groups		
	Total N (%)	*JAK2* V617F positive N (%)	*JAK2* V617F negative N (%)
Essential thrombocythemia	46 (100)	27 (58.70)	19 (41.30)
Polycythemia vera	19 (100)	16 (84.21)	3 (15.79)
Primary myelofibrosis	7 (100)	5 (71.43)	2 (28.57)