Pomeranian J Life Sci 2024;70(1):64-69 doi: 10.21164/pomjlifesci.968

Vascular endothelial growth factor, stromal growth factor 1α and endothelial progenitor cells in essential thrombocythemia – is there a link between angiogenesis and vasculogenesis?

Joanna Boinska[1, A] ✉, Grażyna Gadomska[1, B], Katarzyna Regus[2]

[1] Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Faculty of Pharmacy, Department of Pathophysiology, Sklodowskiej-Curie Street 9, 85-094 Bydgoszcz, Poland
[2] Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Student of the Faculty of Pharmacy, Jagiellońska 15, 85-067 Bydgoszcz, Poland

[A] ORCID: 0000-0001-7649-536X; [B] ORCID: 0000-0003-1917-2809

✉ joanna_boinska@cm.umk.pl

ABSTRACT

Introduction: Angiogenesis and vasculogenesis contribute to the development of cancer.

The aim of the present study was to determine the levels of vascular endothelial growth factor-A (VEGF-A) and stromal cell-derived factor-1α (SDF-1α) and the count of endothelial progenitor cells (EPCs) in patients with essential thrombocythemia (ET).

Materials and methods: The study included a group of 62 patients diagnosed with ET and 25 healthy volunteers. Vascular endothelial growth factor-A and SDF-1α levels were determined by enzyme-linked immunosorbent assays (ELISAs). Endothelial progenitor cells were evaluated by flow cytometry.

Results: The study showed significantly higher levels of both VEGF-A and SDF-1α in ET patients compared to controls (Me = 65.22 pg/mL vs Me = 25.34 pg/mL; Me = 2351.17 pg/mL vs Me = 1742.50 pg/mL, respectively). However, we could not demonstrate a different count of EPCs in ET patients compared to healthy controls. Furthermore, in 40 ET patients with JAK2 V617F mutation (65%), only VEGF-A levels were significantly higher compared to JAK2 V617F negative patients (Me = 72.00 pg/mL vs Me = 53.38 pg/mL). SDF-1α levels were significantly elevated in patients over 60 years of age (Me = 2635.00 pg/mL vs Me = 2395.02 pg/mL). We found positive correlations between VEGF-A and SDF-1α and between VEGF-A and EPCs.

Conclusions: Clinical features such as age over 60 years and Janus 2 kinase (JAK2) mutation may increase angiogenesis in patients with essential thrombocythemia. Despite a positive correlation between VEGF-A and EPCs, the present study suggests weak cooperation between proangiogenic factors and vasculogenesis.

Keywords: essential thrombocythemia; vasculogenesis; endothelial progenitor cells; vascular endothelial growth factor; stromal cell-derived factor-1α.

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic stem cell disorders. They are characterized by abnormal proliferation and growth of erythroid, megakaryocytic, or granulocytic cells [1]. In 2008, the World Health Organization (WHO) identified four classic types of MPNs: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Approximately 95% of adults with CML have leukemia cells with the Philadelphia (Ph) chromosome. For this reason, CML is described as Ph-positive, whereas ET, PV, and PMF are described as Ph-negative [2, 3, 4].

Essential thrombocythemia is an MPN resulting from the clonal transformation of the bone marrow stem cell leading to the proliferation of the megakaryocytic cell line [5, 6]. The reason for increased platelet production by megakaryocytes in ET remains unclear. However, autonomic platelet production, increased sensitivity to cytokines (e.g., interleukin-3), reduced action of platelet inhibitory factors (e.g., transforming growth factor beta), and severe damage to the bone marrow microenvironment may play a role in this mechanism [7].

Most patients with ET have mutations in one of three genes: *Janus 2 kinase* (*JAK2*), *thrombopoietin* gene (*MPL*), or *calreticulin* (*CALR*). The *Janus 2 kinase* mutation, which occurs in approximately 50–60% of patients, can permanently activate the thrombopoietin receptor, leading to the overproduction of megakaryocytes. *Thrombopoietin* gene mutations are associated with approximately 3–5% of ET cases. Somatic mutations in the *CALR* gene are detected in peripheral blood in approximately 25% of *JAK2/MPL*-unmutated ET cases [8].

At the time of ET diagnosis, most patients have no clinical signs or symptoms. Approximately 50% of patients are diagnosed during periodic health assessments or screening tests for a variety of conditions. Other patients present with microcirculatory problems such as paresthesias, headaches, dizziness, blurred vision and painful erythema of the extremities, skin ulcers, and convulsions; 9–22% of patients suffer from large vessel thrombosis, which is the most common complication at the time of diagnosis. Arterial thrombosis can cause stroke or acute coronary syndromes, and venous thrombosis can lead to the development of Budd-Chiari disease or portal vein thrombosis [9, 10].

Angiogenesis, defined as the growth of new blood vessels from pre-existing ones, is a physiological and complex process controlled by the net balance between different biomolecules. The major stimulator of angiogenesis is vascular endothelial growth factor-A (VEGF-A), which promotes endothelial cell proliferation and migration, especially under hypoxic conditions. VEGF-A interacts with several receptor tyrosine kinases, including VEGF receptor 1 (VEGFR-1) and VEGF receptor 2 (VEGFR-2), which are expressed on endothelial cells, bone marrow-derived cells, and cancer cells. In addition, various cytokines or growth factors can upregulate *VEGF* expression. Local or systemic chemical signals coordinate the functions of endothelial and smooth muscle cells to repair damaged blood vessels. Physiological angiogenesis processes are critical during embryonic development, wound healing, and secondary vessel formation to improve organ perfusion. In contrast, aberrantly accelerated angiogenesis is associated with various pathological conditions such as cancer progression, atherosclerosis, diabetes, autoimmune diseases, psoriasis, and many others [11, 12, 13, 14].

Vasculogenesis is defined as the differentiation of angioblasts into endothelial cells and the formation of a new vascular network in both the fetal and postnatal periods [15, 16]. According to recent studies, postnatal vasculogenesis involves endothelial progenitor cells (EPCs) with high proliferative capacity [17]. Cytokines such as VEGF-A, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and stromal cell-derived factor-1α (SDF-1α) are involved in the recruitment of bone marrow EPCs. Interestingly, SDF-1 is a chemotactic cytokine that promotes both VEGF-mediated tumor angiogenesis and EPCs-mediated vasculogenesis [18, 19, 20].

The aim of this study was to evaluate angiogenesis and vasculogenesis in ET patients based on the levels of selected angiogenic cytokines (VEGF-A and SDF-1α) and the number of EPCs in relation to thrombosis risk factors. To our knowledge, a detailed evaluation of these parameters in ET patients has not been reported.

MATERIALS AND METHODS

The study group consisted of 62 patients (F/M 38/24) aged 21–86 years (mean age: 63 ±10 years) with a confirmed diagnosis of ET based on the 2008 WHO criteria and exclusion of other malignant and non-malignant diseases manifested by thrombocythemia. All patients underwent detailed evaluation, including bone marrow biopsy and cytogenetic analysis, necessary for the diagnosis of ET and differential diagnosis with other myeloproliferative neoplasms.

Patients with essential thrombocythemia were newly diagnosed and not previously treated with cytoreductive drugs. All patients were recruited from the Department of Hematology and Malignant Diseases of the Hematopoietic System, University Hospital No. 2 in Bydgoszcz, Poland. Other exclusion criteria were: newly diagnosed thrombotic complications, New York Heart Association (NYHA) functional class III-IV, diabetes, and pregnancy. Of the 62 patients in the study group, 40 had the *JAK2 V617F* mutation (65%). A history of thrombosis such as myocardial infarction, cerebral ischemia, and pulmonary embolism was reported in 10 ET patients (16%); 52 of the patients had no history of thromboembolism (84%); 35 of the 62 patients were over 60 years of age (56.45%).

The control group consisted of 25 healthy volunteers (17 women and 8 men) between 30–65 years of age (mean age: 50 ±12 years). The control group consisted of healthy individuals who underwent medical examinations at the Occupational Medicine Clinic.

The research was approved by the Bioethics Committee No. KB 396/2010.

Samples

Blood samples were collected in the morning between 7:00 and 9:00 am, after the subject had been seated for at least 30 min. Blood was collected by venipuncture into two tubes (Vacutainer® system, Becton Dickinson, Plymouth, UK) containing anticoagulant: 1) dipotassium ethylenediaminetetraacetic acid (K2EDTA), 2) sodium citrate (3.2%).

Complete blood count and endothelial progenitor cell counts were determined in whole blood samples (K2EDTA tubes). Tubes containing sodium citrate were centrifuged at 3000 x g for 15 min. The resulting plasma was pipetted into Eppendorf tubes. The material was stored at –80°C until analysis, but no longer than 6 months. VEGF-A and SDF-1α concentrations were determined in the citrate plasma.

Immunoenzymeatic methods

The following tests were performed using enzyme-linked immunosorbent assay (ELISA) methods: concentration of VEGF-A (R&D Systems Inc., Minneapolis, MN, USA) and SDF-1α (Human CXCL12/SDF-1 alpha, R&D Systems Inc., Minneapolis, MN, USA). All measurements were performed according to the manufacturer's instructions.

Flow cytometry

Quantification of circulating EPCs from peripheral blood was performed using a fluorescence-activated cell sorting (FACS) Calibur flow cytometer (Becton Dickinson, San Diego, CA, USA) with monoclonal antibodies against EPC-specific antigens.

For enumeration of circulating EPCs, fluorescein isothiocyanate (FITC)-conjugated anti-CD31, peridinine-chlorophyll protein-cyanine (PerCP-Cy5. 5)-conjugated anti-CD45, allophycocyanin (APC)-conjugated anti-CD34 antibody (BD Biosciences, Pharmingen, San Diego, CA, USA), and phycoerythrin (PE)-conjugated anti-CD133 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) were used. EPCs were identified by immunophenotype: CD45 (-); CD133 (+); CD31 (+); CD34 (+). A minimum of 100,000 events were measured in each sample. Total cell counts were calculated using TruCount tubes (BD Biosciences, San Jose, CA, USA) containing a calibrated number of fluorescent beads, and lyse-no-wash procedures were used in the present study to improve sensitivity. The absolute number of EPCs (cells/μL) was calculated as follows: number

of EPCs measured/number of fluorescent beads counted × number of beads/μL. The data obtained were analyzed using CellQuest software (Becton Dickinson).

Statistics

Statistical analysis was performed using Statistica 13.3 software (StatSoft, USA). The sample size was estimated using the power analysis module (power: 0.80 (or 80%), alpha level of 0.05). The normality of the variables was tested using the Kolmogorov-Smirnov test.

The results of the normality test in the study group were as follows EPCs: d = 0.22846, p < 0.01; VEGF-A: d = 0.36320, p < 0.01; SDF-1α: K-S d = 0.15432, p < 0.15. The Kolmogorov-Smirnov test indicated that the data were not normally distributed. Therefore, the non-parametric Mann-Whitney U test was used for all comparisons. Data are presented as median and interquartile range. Correlation coefficients were determined by Spearman's test. A probability (p < 0.05) was considered statistically significant.

RESULTS

As shown in Table 1, significantly higher (p < 0.0001) levels of VEGF-A and significantly higher (p < 0.0001) levels of SDF-1α were found in patients with essential thrombocythemia compared to the control group. The number of EPCs was not significantly different between ET patients and controls.

Furthermore, a significantly higher (p < 0.0001) WBC count and a significantly higher (p < 0.0001) PLT count were found in ET patients compared to healthy subjects.

Statistical analysis showed a positive correlation between EPC count and VEGF-A (r = 0.3078, p = 0.0174) as well as VEGF-A concentration and SDF-1α (r = 0.2558, p = 0.0476). There were also positive correlations between VEGF-A and red blood cell count (r = 0.2639, p = 0.0235) and VEGF-A and HCT (r = 0.2618, p = 0.0342). There were also significant relationships between SDF-1α and RBCs (R = 0.3083, p = 0.0225), SDF-1α and HGB (R = 0.3034, p = 0.03371), and SDF-1α and HCT (R = 0.3050, p = 0.0303).

Furthermore, we evaluated parameters of angiogenesis and vasculogenesis in relation to traditional thrombotic risk factors such as the presence of *JAK2V617F* mutation, age over 60 years, and history of thrombosis.

VEGF-A levels were significantly higher in the group of ET patients with the *JAK2 V617F* mutation than in ET patients without this mutation (Tab. 2). However, there was no difference in SDF-1α and EPC counts between ET patients with and without the *JAK2 V617F* mutation.

Among the selected parameters, only SDF-1α concentrations were significantly increased in ET patients above 60 years of age (Tab. 3).

As shown in Table 4, there were no significant differences in all selected parameters in ET patients with and without a history of thrombosis.

TABLE 1. Parameters of angiogenesis and vasculogenesis and complete blood count in patients with essential thrombocythemia (ET) and in the control group

Parameter	Group ET (n = 62) Control (n = 25)	Min	Q1	Me	Q3	Max	p
VEGF-A (pg/mL)	ET	9.78	47.11	65.22	98.99	1390.82	**<0.0001**
	control	7.22	19.60	25.34	27.12	27.64	
SDF-1α (pg/mL)	ET	466.48	1947.00	2351.17	2587.29	4567.70	**<0.0001**
	control	1261.18	1611.03	1742.50	1907.99	2231.48	
EPCs (cells/μL)	ET	0.00	0.20	0.70	2.14	9.87	0.3155
	control	0.00	0.31	0.51	1.02	1.53	
HGB (g/dL)	ET	8.40	13.40	14.35	15.10	16.40	0.1626
	control	12.10	13.00	13.80	14.60	16.20	
HCT (%)	ET	26.20	40.10	42.30	46.00	50.10	0.8997
	control	36.00	40.60	44.20	46.40	47.80	
RBCs (T/L)	ET	2.74	4.64	4.92	5.39	5.97	0.3728
	control	4.01	4.86	5.21	5.42	5.61	
WBCs (G/L)	ET	4.08	8.07	9.78	12.07	16.25	**<0.0001**
	control	4.03	4.76	5.80	7.25	10.00	
PLTs (G/L)	ET	416.00	706.00	873.00	1092.00	2165.00	**<0.0001**
	control	156.00	223.00	256.00	287.00	345.00	

EPCs – endothelial progenitor cells; SDF-1α – stromal cell-derived factor-1α; VEGF-A – vascular endothelial growth factor A; HGB – hemoglobin; HCT – hematocrit; RBCs – red blood cells, WBCs – white blood cells; PLTs – platelets
Significant values are denoted by bold p-values; p-values were determined by the Mann-Whitney U test.

TABLE 2. Assessment of VEGF-A, SDF-1α, and EPCs in ET patients depending on the presence of the *JAK2 V617F* mutation

Parameter	JAK2V617F positive (n = 40) negative (n = 22)	Min	Q1	Me	Q3	Max	p
VEGF-A (pg/mL)	positive	16.94	48.84	72.00	104.07	1390.82	0.0353
	negative	9.78	17.54	53.38	75.66	602.70	
SDF-1α (pg/mL)	positive	561.30	1964.54	2437.51	2650.50	4567.70	0.1109
	negative	466.48	1816.20	2123.65	2387.04	4103.98	
EPCs (cells/μL)	positive	0.00	0.20	0.81	2.84	9.87	0.0713
	negative	0.00	0.20	0.46	0.92	2.95	

EPCs – endothelial progenitor cells; SDF-1α – stromal cell-derived factor-1α; VEGF-A – vascular endothelial growth factor A
Significant values are denoted by bold p-values; p-values were determined by the Mann-Whitney U test.

TABLE 3. Assessment of VEGF-A, SDF-1α, and EPCs in ET patients depending on age

Parameter	> 60 years of age yes (n = 35) no (n = 27)	Min	Q1	Me	Q3	Max	p
VEGF-A (pg/mL)	yes	10.71	42.63	62.98	88.81	1390.82	0.9585
	no	9.78	47.11	63.56	88.26	220.86	
SDF-1α (pg/mL)	yes	808.40	2097.77	2489.20	2666.00	4567.70	0.0399
	no	466.48	1373.80	2272.70	2462.90	2736.20	
EPCs (cells/μL)	yes	0.00	0.20	0.61	1.94	6.62	0.7316
	no	0.00	0.10	0.71	2.85	9.87	

EPCs – endothelial progenitor cells; SDF-1α – stromal cell-derived factor-1α; VEGF-A – vascular endothelial growth factor A
Significant p-values are in bold; p-values were determined by the Mann-Whitney U test.

TABLE 4. Assessment of VEGF-A, SDF-1α, and EPCs in ET patients depending on the past history of thrombosis

Parameter	History of thrombosis yes (n = 10) no (n = 52)	Min	Q1	Me	Q3	Max	p
VEGF-A (pg/m)	yes	28.77	41.57	62.98	194.96	616.91	0.8587
	no	9.78	46.90	64.21	93.90	1390.82	
SDF-1α (pg/mL)	yes	466.48	1174.10	2635.00	2751.50	2970.10	0.4851
	no	561.30	1964.54	2395.02	2599.55	4567.70	
EPCs (cells/μL)	yes	0.00	0.20	1.46	2.14	2.44	0.7483
	no	0.00	0.20	0.65	2.83	9.87	

EPCs – endothelial progenitor cells; SDF-1α – stromal cell-derived factor-1α; VEGF-A – vascular endothelial growth factor A
Significant values are denoted by bold p-values; p-values were determined by the Mann-Whitney U test.

DISCUSSION

The present study showed higher levels of VEGF-A and SDF-1α, as well as higher counts of WBCs and PLTs in patients with ET than in the control group. However, there was no difference in the count of EPCs between ET patients and controls.

Vascular endothelial growth factor-A is the most prominent angiogenic stimulator. It is a selective mitogen for endothelial cells, but also an important factor conditioning their survival and inhibiting apoptosis [21, 22]. It is also known that VEGF-A has a significant impact on postnatal vasculogenesis via the recruitment of bone marrow-derived EPCs. Thus, VEGF-A contributes to the formation of new vessels after ischemia [23, 24, 25]. An analysis of the available literature shows that elevated levels of VEGF-A occur in the blood of patients with hematologic malignancies. Gadomska et al. observed elevated VEGF-A levels in patients with *BCR-ABL* negative myeloproliferative neoplasms, which according to the author may indicate the intensity of neoangiogenesis in the bone marrow [26]. Chen et al. found elevated serum VEGF-A levels in patients with CML [27]. Raimondo et al. found elevated VEGF-A in the blood of patients with primary myelofibrosis [28]. In addition, Treliński et al. and

Alonci et al. demonstrated increased VEGF-A levels in the blood of patients with ET and PV [29, 30]. A review of the available literature indicates that high levels of VEGF-A in patients with various types of myeloproliferative neoplasms are responsible for the intensification of the angiogenesis process.

In the present study, we found elevated levels of SDF-1α in ET patients compared to controls as well as in the subgroup of ET patients over 60 years of age. SDF-1α is a homeostatic chemokine involved in fetal angiogenesis [31]. It is expressed in many organs including lungs, liver, skin and bone marrow. It is responsible for the recruitment of endothelial cells from the bone marrow to sites of vasculogenesis. High levels of SDF1α are synthesized as a result of tissue damage and local hypoxia [32]. Hattori et al. showed that SDF-1α causes the mobilization of hematopoietic cells, such as progenitor and precursor cells [33]. Bae et al. observed the stimulatory effect of SDF-1α on the migration, survival, proliferation, and differentiation of stem cells [34]. Yu et al. demonstrated the cooperation of VEGF-A and SDF-1α in the process of angiogenesis in a mouse model of hind limb ischemia. VEGF stimulated the proliferation of EPCs, and SDF-1α, as a chemoattractant, blocked their apoptosis and increased the number of EPCs at the hypoxic site [35].

In the present study, there was no statistically significant increase in the EPC count in ET patients. Alonci et al. found a slightly increased EPC count in patients with MPN compared to the control group, but the difference was not statistically significant [30].

Stem cell-derived endothelial progenitor cells are essential for the initiation of vasculogenesis because of their ability to self-renew, form colonies, and transform into functional cells. Asahara noted the presence of progenitor cells in the circulation and showed that they are also involved in the formation of new vessels in the postnatal period [17]. The essence of postnatal vasculogenesis is the migration of endothelial progenitor cells from the bone marrow to colonize and differentiate at sites of vascular injury [36].

The current study showed that there was a positive correlation between the number of EPCs and VEGF-A concentration ($r = 0.3078$, $p < 0.05$) as well as a statistically significant positive correlation between VEGF-A and SDF-1α concentration in ET patients ($r = 0.2558$, $p < 0.05$). However, the positive correlation between VEGF-A and the number of EPCs does not seem to be sufficient evidence for a strong relationship between angiogenesis and vasculogenesis. This moderate correlation does not lead to increased EPCs in ET patients.

In the present work, an increased number of WBCs and PLTs was observed in patients with ET. Similar results were obtained by Buxhofer-Ausch et al. in patients with ET. According to the authors, increased platelets and leukocytes are involved in thrombotic complications in the course of ET [37]. Falanga et al. found that excessive activation of platelets and leukocytes can lead to the formation of platelet-leukocyte aggregates, which significantly contribute to the formation of thrombotic events in patients with ET [38,39].

The *JAK2V617F* mutation, discovered in 2005, is a somatic mutation of the tyrosine kinase gene that plays a key role in cellular signaling. The mutation leads to an increase in the enzymatic activity of JAK2 kinase and makes cells sensitive to cytokines. This leads to uncontrolled cell proliferation in myeloproliferative neoplasms, including ET [40]. Almost half of ET cases and according to other data as many as 60–70% of patients are carriers of the *JAK2V617F* mutation [41]. In the present study, we found significantly higher VEGF-A levels in ET patients with *JAK2V617F* mutation compared to patients without this mutation. VEGF-A is the major stimulator of angiogenesis in myeloproliferative neoplasms. Tissue hypoxia is known to be a potent stimulus that activates various genes, including the VEGF gene. The relationship between the *JAK2V617F* mutation and increased VEGF-A levels has been well described by Medinger et al [42] and Medinger and Passweg [43].

In addition, hypoxia is one of the factors that regulate erythropoietin gene expression. In the present study, we found many moderate but significant correlations between both VEGF-A and SDF-1α and erythrocyte count, and HCT and HGB levels. Gadomska et al. also observed a significantly higher number of RBCs in ET patients with the *JAK2V617F* mutation [44]. The present study suggests that a subtle increase in erythropoiesis is observed in patients with essential thrombocythemia. This situation is very similar to vasculogenesis in fetal life, when hemangioblasts differentiate into angioblasts (blood vessel precursors) and blood stem cells. Therefore, the improvement of blood supply results from the formation of new vessels as well as the increase in the number of erythrocytes – oxygen carriers.

Limitations of the study

Several limitations of this study must be acknowledged. First, due to the limited sample size (limited subgroup analyses) and single-center design, our findings should be considered hypothesis-generating and require verification in larger studies. The study group was younger than the control group ($p = 0.039$). Second, the mechanisms of interaction between VEGF-A, SDF-1, and EPCs are still under investigation. More detailed analysis including pro- and anti-angiogenic factors as well as investigation of detailed mechanisms underlying our findings should be considered for future studies.

CONCLUSIONS

Clinical features such as age over 60 years and *JAK2* mutation may increase angiogenesis in patients with essential thrombocythemia. Despite a positive correlation between VEGF-A and EPCs, the present study suggests weak cooperation between proangiogenic factors and vasculogenesis.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391-405.

2. Thiele J, Kvasnicka HM, Orazi A, Tefferi A, Gisslinger H Essential thrombocythaemia. In: Swelderow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon:IARC; 2008. p. 48-50.
3. Bieniaszewska M, Hellmann A. Inne przewlekłe nowotwory mieloproliferacyjne. In: Dmoszyńska A, Robak T, Hus I, editors. Podstawy Hematologii. 3rd ed. Lublin: Czelej. 2015. p. 342-6.
4. Moulard O, Metha J, Fryzek J, Olivares R, Iqbal U, Mesa RA. Epidemiology of myelofibrosis, essential thrombocythemia, and policythemia vera in the Eurpean Union. Eur J Haematol 2013;92(4):289-97.
5. Tefferi A, Vannucchi AM, Barbui T. Essential thrombocythemia treatment algorithm 2018. Blood Canc J 2018;8(1):2.
6. Góra-Tybor J. Nadpłytkowość samoistna. In: Biblioteka czasopisma hematologia 2. Nowotwory mieloproliferacyjne, zespoły mielodysplastyczne i mastocytoza. Gdańsk: Via Medica; 2017. p. 66-75.
7. Prochorec-Sobieszek M, Szumera- Ciećkiewicz A, Szymańska-Giemza O. Diagnostyka różnicowa nadpłytkowości samoistnej. Hematologia 2012;(3):201-10.
8. Rumi E, Pietra D, Ferretti V, Klampfl T, Harutyunyan AS, Milosevic JD, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood 2014;123(10):1544-51.
9. Lal A. Essential thrombocitosis. Medscape; 2017.
10. Hellmann A, Prejzner W. Nowotwory mieloproliferacyjne i mielodysplastyczne. In: Szczeklik A, Gajewski P. Interna Szczeklika 2018. Kraków: Medycyna Praktyczna; 2018. p. 1779-82.
11. Rajabi M, Mousa SA. The role of angiogenesis in cancer treatment. Biomedicines 2017;5(2):34.
12. Folkman J. Tumor angiogenesis therapeutic implications. N Engl J Med 1971;285(21):1182-6.
13. Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. Vasc Health Risk Manag 2006;2(3):213-9.
14. Risau W. Mechanisms of angiogenesis. Nature 1997;386(6626):671-4.
15. Cleaver O, Krieg PA. Vascular development. In: Rosenthal N, Harvey RP, editors. Heart development and regeneration. Academic Press; 2010. p. 487-528.
16. Sadler TW. Langman Embriologia. Malejczyk J, Kujawa M, editors. Wrocław: Edra Urban & Partner; 2017. p. 410.
17. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275(5302):964-7.
18. Murasawa S, Asahara T. Endothelial progenitor cells for vasculogenesis. Physiology 2005;20(1):36-42.
19. Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, et al. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 2001;7(11):1194-201.
20. Laschke MW, Giebels C, Menger MD. Vasculogenesis: a new piece of the endometriosis puzzle. Hum Reprod Update 2011;17(5):628-36.
21. Benjamin LE, Golijanin D, Itin A, Pode D, Keshet E. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J Clin Invest 1999;103(2):159-65.
22. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of Vascular Endothelial Growth Factor (VEGF). J Cell Mol Med 2005;9(4):777-94.
23. Balaji S, King A, Crombleholme TM, Keswani SG. The role of endothelial progenitor cells in postnatal vasculogenesis. Implication for therapeutic neovascularization and wounel healing. Adv Wound Care 2013;2(6):283-95.
24. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011;473(7347):298-307.
25. Ferrara N. VEGF-A: a critical regulator of blood vessel growth. Eur Cytokine Netw 2009;20(4):158-63.
26. Gadomska G, Stankowska K, Boinska J, Ślusarz R, Tylicka M, Michalska M, et al. VEGF-A, sVEGFR-1 and sVEGFR-2 in BCR-ABL negative myeloproliferative neoplasms. Medicina 2017;53(1):34-9.
27. Chen H, Shen YF, Gong F, Yang GH, Jiang YQ, Zhang R. Expression of VEGF and its effect on cell proliferation in patients with chronic myeloid leukemia. Eur Rev Med Pharmacol Sci 2015;19(19):3569-73.
28. Di Raimondo F, Azarro MP, Palumbo GA, Bognato S, Stagno F, Giustolisi GM, et al. Elevated vascular endothelial growth factor (VEGF) serum levels in idiopatic myelofibrosis. Leukemia 2001;15(6):976-80.
29. Trelinski J, Wierzbowska A, Krawczynska A, Sakowicz A, Pietrucha T, et al. Circulating endothelial cells in essential thrombocythemia and polycythemia vera: correlation with JAK2-V617F mutational status, angiogenic factors and coagulation activation markers. Int J Hematol 2010;91(5):792-8.
30. Alonci A, Allegra A, Bellomo G, Penna G, D'Angelo A, Quartarone E, et al. Evaluation of circulating endothelial cells, VEGF and VEGFR2 serum levels in patients with chronic myeloproliferative diseases. Hematol Oncol 2008;26(4):235-9.
31. Ratajczak MZ, Zuba-Surma E, Kucia M, Reca R, Wojakowski W, Ratajczak J, et al. The pleiotropic effects of the SDF-1 – CXCR4 axis in organogenesis regeneration and tumorigenesis. Leukemia 2006;20(1):1915-24.
32. Petit I, Jin D, Rafii S. The SDF-1-CXCR4 signalling pathway: a molecular hub modulating neo-angiogenesis. Trends Immunol 2007;28(7):299-307.
33. Hattori K, Heissig B, Tashiro K, Honjo T, Tateno M, Shieh J-H, et al. Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. Blood 2001;97(11):3354-60.
34. Bae YK, Kim GH, Lee JCH, Seo BM, Joo KM, Lee G, et al. The Significance of SDF-1α-CXCR4 Axis in *in vivo* Angiogenic Ability of Human Periodontal Ligament Stem Cells. Mol Cells 2017;40(6):386-92.
35. Yu JX, Huang XF, Lv WM, Ye CS, Peng XZ, Zhang H, et al. Combination of stromal-derived factor-1α and vascular endothelial growth factor gene-modified endothelial progenitor cells is more effective for ischemic neovascularization. J Vasc Surg 2009;50(3):608-16.
36. Timmermans F, Plum J, Yöder MC, Ingram DA, Vandekeckhove B, Case J. Endothelial progenitor cells: identity defined? J Cell Mol Med 2009;13(1):87-102.
37. Buxhofer-Ausch V, Steurer M, Sormann S, Schloegl E, Schimetta W, Gisslinger B, et al. Influence of platelet and white blood cell counts on major thrombosis - analysis from a patient registry in essential thrombocythemia. Eur J Haematol 2016;97(6):511-6.
38. Falanga A, Marchetti M, Evangelista V, Vignoli A, Licini M, Balicco M, et al. Polymorphonuclear leukocyte activation and hemostasis in patients with essential thrombocythemia and polycythemia vera. Blood 2000;96(13):4261-6.
39. Falanga A, Marchetti M, Vignoli A, Balducci D, Barbui T. Leukocyte-platelet interaction in patients with essential thrombocythemia and polycythemia vera. Exp Hematol 2005;33(5):523-30.
40. Dzietczenia J, Kuliczkowski K. The role of JAK2 in essential thrombocythemia. Acta Haematol Pol 2007;38(20):187-94.
41. Nangalia J, Green AR. Myeloproliferative neoplasms: from origins to outcomes. Blood 2017;130(23):2475-83.
42. Medinger M, Skoda R, Gratwohl A, Theocharides A, Buser A, Heim D, et al. Angiogenesis and vascular endothelial growth factor-/receptor expression in myeloproliferative neoplasms: correlation with clinical parameters and JAK2-V617F mutational status. Br J Haematol 2009;146(2):150-7.
43. Medinger M, Passweg J. Angiogenesis in myeloproliferative neoplasms, new markers and future directions. Memo 2014;7:206-10.
44. Gadomska G, Bartoszewska-Kubiak A, Boinska J, Matiakowska K, Ziołkowska K, Haus O, et al. Selected parameters of angiogenesis and the JAK2, CALR, and MPL mutations in patients with essential thrombocythemia. Clin Appl Thromb Hemost 2018;24(7):1056-60.