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Grażyna Gadomska[1], Klaudia Chałupnik[2], Katarzyna Stankowska[2], Joanna Boinska[2], Ewelina Drela[2], Barbara Ruszkowska-Ciastek[2], Monika Leszcz[2], Jacek Kubica[3], Danuta Rość[2]

ENDOTHELIAL PROGENITOR CELLS IN MYELOPROLIFERATIVE NEOPLASMS – PRELIMINARY REPORT

KOMÓRKI PROGENITOROWE ŚRÓDBŁONKA W NOWOTWORACH MIELOPROLIFERACYJNYCH – DONIESIENIA WSTĘPNE

[1]Department of Hematology and Malignant Diseases of Hematopoietic System,
Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Poland
Head of Department: dr hab. n. med. Jarosław Czyż
[2]Department of Pathophysiology, Nicolaus Copernicus University in Toruń,
Collegium Medicum in Bydgoszcz, Poland
Head of Department: prof. dr hab. n. med. Danuta Rość
[3]Department of Cardiology and Internal Medicine,
Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland
Head of Department: prof. dr hab. n. med. Jacek Kubica

Summary

The aim of this study was to assess the number of endothelial progenitor cells in patients with chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET). The study involved 21 patients (mean age 61.77) with myeloproliferative neoplasms, hospitalized and diagnosed at the Hematology Clinic of Dr. J. Biziel University Hospital No. 2 in Bydgoszcz, Poland.

Material and methods. The study group included 12 patients with ET, 5 with PV, 4 with CML. The control group consisted of 25 healthy volunteers, age- and sex- matched. The material for the study was venous blood collected from the elbow vein into tube containing K2EDTA.

The number of endothelial progenitor cells was measured with FACSCalibur flow cytometer (Becton Dickinson, San Diego, USA) using monoclonal antibodies directed against antigens specific for endothelial progenitor cells (EPCs).

Results. We observed significantly increased number of EPCs in patients with myeloproliferative neoplasms (MPNs) in comparison to the control group. Detailed analysis showed slightly higher number of EPCs in patients with PV and ET than in the controls, but the differences were not statistically significant. The highest statistically significant number of EPCs was observed in patients with CML.

Conclusions. Increased number of EPCs in patients with myeloproliferative neoplasms may indicate increased angiogenesis in these diseases and participation of EPCs in the process of neovascularization.

Streszczenie

Celem niniejszej pracy była ocena liczby i funkcji komórek progenitorowych śródbłonka w przewlekłej białaczce szpikowej (PBS), czerwienicy prawdziwej (CzP), nadpłytkowości samoistnej (NS). Badaniem objęto 21 pacjentów z nowotworami mieloproliferacyjnymi (średnia wieku 61,77), hospitalizowanych w Oddziale Klinicznym Hematologii i Chorób Rozrostowych Układu Krwiotwórczego Szpitala Uniwersyteckiego nr 2 im. Jana Biziela w Bydgoszczy.

Materiał i metody. Badania przeprowadzono u 12 chorych na ET, 4 chorych na CML i 5 chorych na PV. Grupę kontrolną stanowiło 25 zdrowych ochotników. Materiałem do badań była krew pobrana w godzinach porannych z nakłucia żyły łokciowej do probówki

zawierającej wersenian dwupotasowy (EDTA). Po inkubacji z odpowiednimi odczynnikami dokonana została analiza cytometryczna przy użyciu cytometru przepływowego FACS Calibur (Becton Dickinson, San Diego, USA) z zastosowaniem programu komputerowego CellQuest.

W y n i k i . U chorych na przewlekłe nowotwory mieloproliferacyjne stwierdzono istotnie statystyczną zwiększoną liczbę EPCs w porównaniu z grupą kontrolną. U chorych z PV i ET stwierdzono nieznacznie zwiększoną liczbę EPCs w porównaniu do grupy kontrolnej, a różnica ta okazała się nieistotna statystycznie. Najwyższą liczbę EPCs stwierdzono u pacjentów z CML i różnica ta była istotna statystycznie.

W n i o s k i . Zwiększenie liczby EPCs w grupie chorych na przewlekłe nowotwory mieloproliferacyjne świadczy o aktywacji procesów angiogenezy w tych nowotworach i prawdopodobnie czynnym udziale tych komórek w procesie nowotworzenia naczyń.

Key words: endothelial progenitor cells, myeloproliferative neoplasms
Słowa kluczowe: komórki progenitorowe śródbłonka, nowotwory mieloproliferacyjne

INTRODUCTION

Myeloproliferative disorders (MPDs), which were described in a 2008 World Health Organization (WHO) classification as 'myeloproliferative neoplasms' (MPNs), are a group of diseases characterized by clonal proliferation of one or more myeloid cell lines caused by genetic alterations of multipotent stem cells [1, 2]. 'Classic myeloproliferative neoplasms' include: chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).

The first concept of myeloproliferative neoplasms is credited to Dameshek who assumed that these diseases result from disturbances of mechanisms regulating normal hematopoiesis [3, 4]. Starting from the discovery of the Philadelphia chromosome (Ph) in 1960, and then thanks to research on glucose-6-phosphate dehydrogenase (G6PD) isoenzyme phenomenon conducted by Fiałkowa in the 70s of the last century and introduction of clonogenic assays by Bradley and Metcalf, all of the above proved that at the foundation of MPNs lies mutation of multipotential stem cells [3, 5].

Endothelial progenitor cells (EPCs), derived from bone marrow, represent a heterogeneous group of blasts capable of self-renewal, colony-forming and differentiation 'on demand' into functional cells [6, 7, 8]. EPCs were first described by Asahara et al. in 1997 [9]. This discovery changed the view on vasculogenesis. Previously it had been believed that new vessels arose from undifferentiated angioblasts or endothelial progenitor cells during embryogenesis. Research conducted by Asahara et al. showed that EPCs participate in postnatal vasculogenesis [9, 10]. Endothelial progenitor cells are secreted into the blood as a result of endothelial damage for example by inflammatory factors, cytokines, or circulating autoantibodies [11, 12].

Recent years have seen an increase in interest in the subject of participation of endothelial progenitor cells in the pathogenesis of solid tumors – their growth depends on the development of new vascular networks. The newly formed blood vessels supply sufficient amounts of oxygen and nutrients to the growing tumor. Studies of patients with non-small cell lung cancer showed an increased number of EPCs in the endothelial tubes of tumor capillaries [13]. Participation of EPCs in formation of tumor vessels was confirmed in several solid tumors such as malignant glioma, lung cancer and breast cancer [14, 15]. However, the involvement of EPCs in angiogenesis is still under investigation [15].

Hematological malignancies do not develop as a compact tumor mass, for which a vascularization is a critical factor for further growth, invasion and metastasis. Tumor cells in myeloproliferative neoplasms arise in bone marrow and in the absence of the bone marrow-blood barrier they penetrate various organs. Researchers found that in patients with multiple myeloma, acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndromes and primary myelofibrosis there is an increased vascularization of bone marrow and increased expression of angiogenic factors such as VEGF, bFGF, GM-CSF, G-CSF, EPO, and PlGF SDF [16]. These cytokines, besides controlling the formation of blood vessels, can also affect hematopoiesis and bone marrow stromal cells [16, 17, 18].

The aim of this study was to assess the number of endothelial progenitor cells in patients with chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET).

MATERIAL AND METHODS

The study involved 21 patients (mean age 61.77) with myeloproliferative neoplasms, hospitalized and diagnosed at the Hematology Clinic of Dr. J. Biziel University Hospital No. 2 in Bydgoszcz, Poland. These patients were enrolled in the study at the time of the

diagnosis of MPNs and prior to the implementation of an appropriate treatment. The diagnosis was based on the diagnostic criteria of myeloproliferative neoplasms according to the WHO (2008) including: medical interview, physical examination and additional tests (complete blood count with peripheral blood smear, selected parameters of coagulation system, bone marrow biopsy with histopathological evaluation and cytogenetics). The study group included 12 patients with essential thrombocythemia (mean age 59.25), 5 with polycythemia vera (mean age 65) and 4 with chronic myeloid leukemia (mean age 66). The control group consisted of 25 healthy volunteers (mean age 42.8).

The study obtained the approval of the local Ethics Committee (KB 396/2010). Each studied person was informed about the purpose of the research and gave written consent.

The number of endothelial progenitor cells was measured with FACSCalibur flow cytometer (Becton Dickinson, San Diego, USA) using monoclonal antibodies directed against antigens specific for endothelial progenitor cells (EPCs). Acquired data were analyzed by using CellQuest software (Becton Dickinson).

The following monoclonal antibodies were used in this study: fluorescein isothiocyanate (FITC) – conjugated anti-CD31, PerCP-Cy5.5-conjugated anti-CD45, as well as APC-conjugated anti-CD34 antibody (all BD Biosciences, Pharmingen, San Diego, CA, USA), phycoerythrin (PE)-conjugated anti-CD133 (Miltenyi Biotec, Bergisch Gladbach, Germany). Endothelial progenitor cells (EPCs) were defined as negative for the hematopoietic marker CD 45 and positive for the endothelial progenitor marker CD 133, and positive for the endothelial cell markers CD 31 and CD 34. At least 100 000 events were collected before analysis. 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.

The statistical analysis was performed using Statistica 10 software (StatSoft®). Shapiro-Wilk test was used to assess the normality of distribution. Since the examined parameter showed a non-normal distribution, median (Me) and quartiles (lower - Q1 and upper - Q3) were used. Mann Whitney U test were used to compare the difference between groups. The p-values <0.05 were considered significant.

RESULTS

Table I shows the number of endothelial progenitor cells (EPCs) in MPNs patients and in the control group. We observed significantly higher number of EPCs in patients with myeloproliferative neoplasms than in the controls.

Table I. *Comparison of the number of endothelial progenitor cells (EPCs) in patients with myeloproliferative neoplasms and in the control group*

Tabela I. *Porównanie liczby komórek progenitorowych śródbłonka (EPCs) w grupie kontrolnej i chorych na nowotwory mieloproliferacyjne*

Parameter Parametr	Study group Grupa badana n=21			Control group Grupa kontrolna n=25			p
	Me	Q1;Q3	Min-Max	Me	Q1;Q3	Min-Max	
EPCs/µL	0.97	0.51;2.83	0.00-8.96	0.51	0.31;1.02	0.00-1.53	**0.035202**

Table. II. *Number of endothelial progenitor cells (EPCs) in PV, ET and CML patients compared to the control group*

Tabela II. *Liczba komórek progenitorowych śródbłonka w grupie chorych na CzP, NS i PBS w porównaniu do grupy kontrolnej*

EPCs/µl	**PV CzP**	**ET NS**	**CML PBS**	**CONTROL GROUP Grupa kontrolna (C)**	**p**
N	5	12	4	25	PV vs C p=0,09 ET vs C p=0,12 CML vs C p=**0.009513**
ME Q1;Q3	0.97 0.41;2.34	0.70 0.41;2.59	3,56 1.73; 4.96	0.61 0.31;0.92	
Min-Max	0.10 – 8.96	0.00 – 6.01	0.92; 5.33	0.00 – 1.63	

Moreover, a detailed analysis in subgroups of patients with MPNs showed that a significantly higher number of EPCs was found in patients with CML than in the control group (Table II, Figure 1).

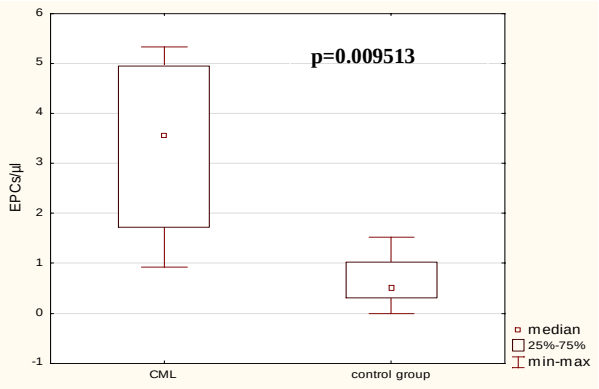

Fig. 1. *Number of endothelial progenitor cells (EPCs) in patients with chronic myeloid leukemia CML compared to the control group*

Ryc. 1. *Porównanie liczby komórek progenitorowych śród-błonka (EPCs) w grupie kontrolnej i grupie chorych na przewlekłą białaczkę szpikową (PBS)*

DISCUSSION

Angiogenesis is a multistep process involved in tumor growth and metastasis formation [19]. Angiogenesis is regulated by many angiogenic factors, among them: vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), granulocyte colony-stimulating factor (G-CSF), interleukin-1 (IL-1), interleukin-6 (IL-6), angiopoietin 1 and 2 [20].

Recently there has been an increased interest in the role of endothelial progenitor cells in the context of angiogenesis, especially in hematological malignancies.

Endothelial progenitor cells (EPCs) represent a heterogeneous group of blasts capable of self-renewal, colony-forming and differentiation 'on demand' into functional cells. It has been shown that several factors may lead to release of EPCs from bone marrow into circulation, where EPCs migrate to the site of injury in vascular system [8, 21]. However, mechanism of EPCs migration and homing remains unclear. It is known that the most potent chemoattractant for EPCs is the stromal cell-derived factor-1 (SDF-1) [21].

The aim of this study was to assess the number of endothelial progenitor cells in patients with myeloproliferative neoplasms. Analysis of the results obtained in the present study showed that the number of EPCs was significantly increased in patients with myeloproliferative neoplasms in comparison to the control group. The highest number of EPCs was observed in patients with CML. However, in patients with PV and ET the number of EPCs was slightly increased compared to the control group but the differences were not statistically significant.

Rafat et al. examined the relationship between the number of EPCs and angiogenesis in patients with malignant gliomas. Researchers observed significantly higher mobilization of EPCs in these patients than in the control group. In addition, Rafat et al. showed significant correlations between EPCs number and serum level of VEGF, as well as between EPCs and vessel density, and a negative correlation between the number of EPCs and tumor size. Patients with malignant gliomas and high density of tumor blood vessels have a higher level of EPCs than patients with a low vessel density [22]. The relationship between the number of EPCs and angiogenesis were also reported by Arbab et al., Lin et al., and Deng et al. Researchers have demonstrated that patients with non-small cell lung cancer, breast cancer and hepatocellular carcinoma have a significantly higher level of EPCs in comparison with the control group. Moreover, they found significant correlations between EPCs number and tumor microvessel density, and between EPCs and VEGF level in these patients [13, 14, 15, 23].

The assessment of angiogenesis process is based primarily on measurement of microvessel density in bone marrow and on the evaluation of VEGF concentration. However, for the assessment of bone marrow microvessel density, invasive diagnostic procedures such as biopsy of the tumor or bone marrow are necessary. There is an ongoing search for non-invasive, effective research methods to assess the intensity of angiogenesis in the course of many diseases, including in myeloproliferative neoplasms [8, 24].

The present study showed slightly higher number of EPCs in patients with PV and ET than in the controls, but the differences were not statistically significant.

Alonci et al. observed significantly elevated EPCs number in patients with PV. Leibundgut et al. reported significantly increased number of endothelial progenitor cells in patients with PV and ET [25, 26].

Summing up, results obtained in the present study, as demonstrated by the higher number of EPCs found in patients with myeloproliferative neoplasms compared to the controls, indicate that endothelial progenitor cells are mobilized from bone marrow into peripheral blood. It is known from the literature that EPCs promote angiogenesis [15]. However, due to the small size of the group of patients with MPNs and the uneven distribution of the number of patients with various myeloproliferative neoplasms, current results are considered preliminary and research is being continued on a larger number of patients with MPNs.

CONCLUSIONS

We observed significantly higher number of EPCs in patients with myeloproliferative neoplasms than in the control group; the highest number of EPCs was noted in patients with CML.

Increased number of EPCs in patients with myeloproliferative neoplasms may indicate increased angiogenesis in these diseases and participation of EPCs in the process of neovascularization.

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Address for correspondence:

Katarzyna Stankowska

Department of Pathophysiology, Nicolaus Copernicus University in Toruń

Collegium Medicum in Bydgoszcz

Marii Skłodowskiej-Curie 9

85-094 Bydgoszcz, Poland

e-mail: stankowska_katarzyna@wp.pl

tel. 52/585-35-91

fax 52/585-35-95

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