ORIGINAL PAPERS

Adv Clin Exp Med 2015, **24**, 6, 987–994
DOI: 10.17219/acem/33811

ISSN 1899–5276

Małgorzata Kobusiak-Prokopowicz[A–D], Beata Jołda-Mydłowska[A–C], Tomasz Grzebieniak[A, B], Karol Początek[A, B], Andrzej Mysiak[E, F]

Expression of Proinflammatory Factors, Proangiogenic Factors and Endostatin in Patients with Heart Failure and Different Grades of Collateral Circulation Development*

Department and Clinic of Cardiology, Wroclaw Medical University, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. The process of collateral vessel maturation is stimulated by numerous factors affecting the endothelium and smooth muscle cells building the vessel wall. Looking for arteriogenesis stimulating factors means looking for a potential innovative heart failure treatment method in the patients unresponsive to traditional therapies.
Objectives. The purpose of this study was to assess the changes in serum concentrations of pro-inflammatory factor IL-6, growth factors FGF (FGFa, FGFb, FGFbH), HGF, VEGF and endostatin in heart failure patients in relation to the coronary collaterals development stage.
Material and Methods. This study included 22 patients with chronic heart failure NYHA II or III (mean age 62.5 ± 11.6 years) and 8 control patients (mean age 58.4 ± 10.7 years). Coronary angiography was performed and the presence and grade of collateral circulation was assessed by a four-level scale proposed by Rentrop and Cohen. The level of the studied factors was determined in the blood samples collected during the angiographic procedure.
Results. The concentration of IL-6 was significantly higher in the heart failure patients than in the control group ($p < 0.001$) and in NYHA III vs. NYHA II patients ($p < 0.02$). Patients with heart failure and collaterals grade 1 or 2 exhibited higher serum concentrations of FGFbH (from $p < 0.03$ to $p < 0.01$). The serum VEGF level in NYHA III patients was significantly higher than in NYHA II individuals (from $p < 0.03$ to $p < 0.01$).
Conclusions. Higher levels of IL-6 and FGFbH were observed in patients with heart failure. Collaterals formation seems to be associated with the activation of pro-inflammatory factors, growth factors and endostatin (**Adv Clin Exp Med 2015, 24, 6, 987–994**).

Key words: arteriogenesis, proinflammatory factors, proangiogenic factors, endostatin, innovative treatment.

In patients with heart failure, regardless of its cause, cardiac hypertrophy and, usually disproportionate, neovascularization are observed. Coronary microvascular dysfunction with subsequent ischemia intensify the process of myocardial damage, the inhibition of which potentially depends on the development of the "vascular tree" and its ability to protect tissues from ischemia [1].

There are three basic processes of new blood vessel development (neovascularization). The first one involves the formation of arterial collaterals (arteriogenesis), the second is based on the sprouting of capillaries (angiogenesis) and the third is the formation of blood vessels by *de novo* differentiation and proliferation of endothelial cells from stem cells (vasculogenesis) [2, 3].

Arteriogenesis is the formation of mature blood vessels from pre-existing capillaries *via* enlarging of the lumen and thickening of the walls. Collateral vessels, developed during arteriogenesis, are large epicardial arteries that allow for efficient blood flow. Formation of these vessels is strictly

* The paper expenses were covered by statutory sources – grant no. 1749.

limited to the area surrounding the narrowed or blocked epicardial artery. This is confirmed by angiographic images of collateral bypasses in patients with advanced occlusion of the main arteries. The role of arteriogenesis in collateral circulation development is important in ischemic and hypoxic organs affected by atherosclerosis. When an occlusion occurs in an existing vessel, blood is directed into other vessels, filling all the patent, but not fully developed vessels. The process of their maturation is stimulated by a number of factors affecting the endothelium and smooth muscle cells of the vessel wall. The best conditions for collaterals formation involve increased fluid shear stress and stimulation of bone marrow-derived cells [4]. In the course of arteriogenesis, perivascular macrophages produce monocyte chemotactic protein MCP-1 and basic fibroblast growth factors, bFGF and VEGF, which are important mediators of arteriogenesis, but probably to a lesser degree than angiogenesis [5, 6].

Angiogenesis results in the formation of small, intramyocardially spreading vessels with thin walls. Collaterals formed in the process of arteriogenesis exhibit low resistance, while angiogenesis results in the formation of a high-resistance vascular bed. Angiogenesis is dependent on the interaction of endothelial cells, angiogenic mediators, cytokines, growth factors and cell adhesion molecules [7]. Many studies have shown that inflammation may also contribute to the formation of new blood vessels. The influx of inflammatory cells such as macrophages, monocytes and platelets, leads to the release of numerous cytokines that stimulate the release of proangiogenic factors [8].

Chung et al. showed that the levels of angiogenic factors and thus the development of collateral circulation were greater in patients with chronic ischemic heart disease [9]. In patients with ischemic heart disease who developed collateral circulation, the level of anti-angiogenic factors was 40% lower than in patients lacking the collaterals [10].

With this group of patients in mind, many research centers are striving to develop alternative methods of restoring coronary vasculature. Collateral circulation plays an immensely important role in limiting the ischemic zone and myocardial infarction size. Charney and Cohen observed patients undergoing coronary angioplasty and found that transient myocardial ischemia was better tolerated in patients with collateral flow than in those without. The collateral circulation also reduced the risk of perioperative infarct rate and death during coronary artery bypass grafting [11]. Looking for neovascularization stimulating factors is, in fact, looking for a potential innovative treatment method, especially for those patients in whom traditional invasive surgical and pharmacological therapy is ineffective [12].

Aim of the Study

The purpose of this study was to assess changes in serum concentrations of pro-inflammatory factor IL-6, growth factors FGF (FGFa, FGFb, FGFbH), HGF and VEGF and endostatin in patients with heart failure in relation to the coronary collaterals development stage.

Material and Methods

This study included 22 patients admitted to the Department of Cardiology, Wroclaw Medical University, with a diagnosis of chronic heart failure NYHA stage II or III (mean age 62.5 ± 11.6 years), including 8 women aged 49–81 years (mean age 69.26 ± 9.5 years) and 14 men aged 41–86 years (mean age 60.0 ± 11.3 years). Twelve patients were diagnosed with heart failure due to ischemic heart disease and 10 patients suffered from idiopathic dilated cardiomyopathy. In the group of patients with heart failure, the mean left ventricular ejection fraction (LVEF) was 33.5% ± 7.4%. The control group consisted of 8 age-matched participants, 2 women and 6 men (mean age: 58.4 ± 10.7 years), without heart failure, diagnosed for ischemic heart disease. The mean LVEF in this group was 57.5% ± 2.7%.

The study excluded individuals with acute renal failure and stages 4 and 5 of chronic kidney disease, thyroid disease, neoplastic disease, acute inflammatory disease, treatment history of steroids and immunosuppressive drugs and extremely high blood glucose levels exceeding 400 mg/dL.

The study protocol was granted the consent of the local Ethics Committee and research was conducted in accordance with the Declaration of Helsinki.

Coronary angiography of the studied groups was performed using a standard method of puncturing the femoral artery. To appropriately visualize the coronary arteries, a contrast medium was selectively administered to the left coronary artery, followed by the right coronary artery. Injection recordings were carried out in standard projections (LAO 60° and 90°, RAO 30°) and supplemented where necessary with additional injections for imaging distal or overlapping fragments of vessels. If any atherosclerotic lesions were found, vessel stenosis was assessed based on digital image analysis of a QCA (Quantitative Coronary Angiography) image. Stenosis exceeding 60% was deemed angiographically significant.

Table 1. Rentrop scale and number of vessels with collaterals

Scale	Characteristics of collateral circulation
0	no visible filling of any vessel
1	visible filling of the extended branches of the collaterals circulation, but not the epicardial portion of the artery 17 patients – 0 vessels 2 patients – 1 vessel 3 patients – 2 vessels
2	visible collateral circulation filling the distal portion of the epicardial artery 17 patients – 0 vessels 4 patients – 1 vessel 1 patient – 2 vessels
3	complete collateral filling of the epicardial artery 20 patients – 0 vessels 2 patients – 1 vessel

The presence and grade of collateral circulation were assessed by the four-level scale proposed by Rentrop and Cohen [13, 14]. Angiograms were evaluated by two doctors without access to clinical data (Table 1).

To determine the level of the studied factors, blood samples were collected from each patient during the angiographic procedure. Blood samples were taken from the femoral artery (sample 1), the left main coronary artery or the initial segment of the LAD (sample 2) and a peripheral vein (sample 3). After collecting blood into SST tubes, the samples were left for 30 min for clotting. The samples for IL-6, VEGF, FGFa, FGFb, FGFbH and endostatin were then centrifuged for 15 min at 1000 × g and the serum was stored at ≤ –20° C. HGF samples were centrifuged for 10 min at 1000 × g and the serum was stored at ≤ –70° C.

The concentration of IL-6 was determined using ELISA assay (R&D Systems, Inc., Catalog Number DHG00); inter-assay precision was 1.6–4.2% and intra-assay precision was 3.3–6.4%. The concentration of VEGF was determined using ELISA assay (R&D Systems, Inc., Catalog Number DVE00); inter-assay precision was 4.5–6.7% and intra-assay precision was 6.2–8.8%. The concentration of endostatin was determined using ELISA assay (R&D Systems, Inc., Catalog Number DNST0); inter-assay precision was 3.6–6.9% and intra-assay precision was 5.7–7.9%. The concentration of FGF basic high sensitivity (HFGFb) was determined using ELISA assay (R&D Systems, Inc., Catalog Number HSFB00D); inter-assay precision was 3.5–7.7% and intra-assay precision was 4.7–8.2%. The concentration of FGF basic (FGFb) was determined using ELISA assay (R&D Systems, Inc., Catalog Number DFB50); inter-assay precision was 3.0–9.7% and intra-assay precision was 7.4–9.1%. The concentration of FGF acidic (FGFa) was determined using ELISA assay (R&D Systems, Inc., Catalog Number DFA00B); intra-assay precision was 2.3–7.2% and inter-assay precision was 8.4–8.6%. The concentration of HGF was determined using ELISA assay (R&D Systems, Inc., Catalog Number DHG00); inter-assay precision was 4.1–7.0% and intra-assay precision was 5.4–8.4%.

Within 48 h after admission, trans-thoracic echocardiography was performed in all of the patients using a Vingmed System 5, General Electric device equipped with a 2.5 MHz probe. The EF was determined using the standard formula: EF = (LVEDV - LVESV)/LVEDV × 100%, where LVEDV is left ventricular end-diastolic volume and LVESV is left ventricular end-systolic volume. LVEDV and LVESV were calculated using the biplane Simpson method, in which a computer determined the left ventricular volume using approximately perpendicular cross-section areas of the left ventricle in apical two- and four-chamber views, determined by an investigator.

Statistical Analysis

Calculations were made using the statistical software, STATISTICA v. 5.0. Measurable analyzed parameters were characterized using the arithmetic mean and standard deviation. For parameters whose normal distribution was confirmed by the Shapiro-Wilk test, the differences between the two groups were assessed using Student's *t*-test for independent variables, after testing equality of variances with the Fisher-Snedecor test. When normal distribution was not confirmed, the nonparametric Mann-Whitney *U* test or ANOVA Kruskal-Wallis rank test was used.

The assumed level of statistical significance was $p < 0.05$.

Results

The results are shown in Tables 2–4.

The concentration of IL-6 in the peripheral blood (sample 1 and sample 3) was significantly higher in the heart failure patients than in the

Table 2. Biochemical parameters in patients with heart failure (HF) and in the control group

Biochemical parameter		HF group n = 22	Control group n = 8	Significance of differences – p
IL6 (1) pg/mL	mean SD	11.54 19.78	1.95 1.42	0.001
IL6 (2) pg/mL	mean SD	12.32 22.06	15.19 34.61	ns.
IL6 (3) pg/mL	mean SD	10.92 20.71	3.25 5.23	0.001
Endostatin (1) ng/mL	mean SD	140.53 35.46	132.32 32.80	ns.
Endostatin (2) ng/mL	mean SD	143.39 50.25	129.36 31.30	ns.
Endostatin (3) ng/mL	mean SD	141.95 37.66	137.63 38.23	ns.
FGFb (1) pg/mL	mean SD	4.64 4.80	4.66 4.77	ns.
FGFb (2) pg/mL	mean SD	8.91 9.24	5.35 6.02	ns.
FGFb (3) pg/mL	mean SD	3.25 2.47	2.83 2.34	ns.
FGFa (1) pg/mL	mean SD	17.02 13.72	17.85 6.08	ns.
FGFa (2) pg/mL	mean SD	17.70 12.41	22.00 7.87	ns.
FGFa (3) pg/mL	mean SD	19.11 15.21	19.17 9.04	ns.
FGFbH (1) pg/mL	mean SD	3.80 2.11	1.60 0.56	0.01
FGFbH (2) pg/mL	mean SD	3.40 1.73	2.08 1.52	0.03
FGFbH (3) pg/mL	mean SD	3.28 2.10	1.73 1.48	0.01
HGF (1) pg/mL	mean SD	1323.79 429.32	1180.12 474.39	ns.
HGF (2) pg/mL	mean SD	1561.83 489.25	2434.39 307.91	ns.
HGF (3) pg/mL	mean SD	1571.90 628.89	1175.99 259.81	ns.
VEGF (1) pg/mL	mean SD	297.04 286.35	178.78 61.06	ns.
VEGF (2) pg/mL	mean SD	270.94 242.24	166.05 70.70	ns.
VEGF (3) pg/mL	mean SD	311.32 254.81	164.02 86.07	ns.

Table 3. Biochemical parameters in patients with NYHA II and III

Biochemical parameter		HF NYHA II n = 13	HF NYHA III n = 9	Significance of differences – p
IL6 (1) pg/mL	mean SD	4.23 3.24	23.42 28.56	0.02
IL6 (2) pg/mL	mean SD	7.89 10.70	19.58 28.98	0.02
IL6 (3) pg/mL	mean SD	7.17 10.98	16.35 26.20	0.02
VEGF (1) pg/mL	mean SD	192.43 147.34	467.02 379.01	0.02
VEGF (2) pg/mL	mean SD	171.60 138.29	432.36 294.56	0.01
VEGF (3) pg/mL	mean SD	218.14 172.94	445.91 301.80	0.03

Table 4. Biochemical parameters and the presence of vascular collaterals in HF patients (ANOVA Kruskal-Wallis rank test)

Biochemical parameter	Median	Significance of differences – p
IL-6 (2) pg/mL	3.07	0.02
IL-6 (3) pg/mL	2.65	0.05
Endostatin (1) ng/mL	129.00	0.05
Endostatin (2) ng/mL	132.60	0.05
FGFbH (2) pg/mL	4.59	0.05
FGFbH (3) pg/mL	3.41	0.05
HGF (1) pg/mL	1165.74	0.05
HGF (3) pg/mL	1358.13	0.05
VEGF (2) pg/mL	129.00	0.05
VEGF (3) pg/mL	294.57	0.05

control group ($p < 0.001$). In addition, IL-6 serum levels in all 3 studied blood samples were significantly higher in patients with NYHA III HF as compared to the NYHA II group ($p < 0.02$). The serum IL-6 concentration was, however, significantly lower in patients with collaterals grade 1 or 2, as assessed by the Rentrop scale ($p < 0.03$ and $p < 0.04$).

Patients with heart failure exhibited higher serum concentrations of FGFbH (from $p < 0.03$ to $p < 0.01$). The serum concentration of FGFbH was also significantly higher in patients with collaterals grade 1 or 2, as assessed by the Rentrop scale ($p < 0.02$).

The serum levels of endostatin, FGFb, FGFa, VEGF and HGF did not differ significantly in any of the samples for the patients with heart failure and the control group. However, the serum VEGF level in NYHA III patients was significantly higher than in NYHA II individuals (from $p < 0.03$ to $p < 0.01$).

The ANOVA Kruskal-Wallis rank test revealed significant correlations between the levels of IL-6, endostatin, FGFbH, HGF and VEGF and the presence of collateral vessels.

Discussion

The main arteriogenesis-inducing factor is increased shear stress in vessels and the result is the formation of collateral arteries far from the ischemic area [15]. The acute phase of the systemic response is mediated by proinflammatory cytokines, particularly IL-1 and IL-6, acting in a complex manner [16]. Proinflammatory cytokines, produced within inflammation areas [17, 18], are potent autocrine and paracrine agents and may affect vascular function [19, 20].

IL-6 was suspected of exhibiting angiogenic activity, as high levels of this interleukin were determined within the areas of the endothelium where enhanced angiogenesis was observed. Such processes take place, for example, during wound healing or in carcinogenesis (Kaposi's sarcoma) [21]. Aoki et al. published the results of an experiment in which they inoculated athymic mice with cells transformed to secrete IL-6 and found that they stimulated the growth of more vascularized tumor-like lesions [21].

The mechanisms by which IL-6 stimulates angiogenesis are complex, but a number of growth factors with known angiogenic properties, such as HGF, EGF and VEGF, stimulate the activation of

STAT3 factor (signal transducer and transcription 3 factor) [22, 23].

The ability of IL-6 to induce full angiogenic activity in *ex vivo* and *in vivo* models was also tested [17]. It was hypothesized that IL-6 may directly affect individual components of the vascular wall, thus contributing to the prevention of ischemic episodes. Our study compared the release of IL-6 and collaterals formation during the arteriogenesis process. In patients with heart failure, IL-6 serum concentrations in peripheral arteries and veins were significantly higher than in healthy subjects. Levels of IL-6 were also significantly greater in all vascular territories in patients with HF and higher NYHA classes than in those with developed collateral circulation. Our observations revealed that the role of IL-6 in the pathophysiology of heart failure was not associated with arteriogenesis progress, despite its expected effect on the vascular wall.

VEGF is a potent mobilizer of stem cells and progenitor cells. In the study by El-Melegy et al., significantly higher VEGF levels were found in children with congenital heart disease and developed collaterals [24]. Patel et al. reported that serum VEGF levels did not correlate with objective markers of left ventricular function, but were significantly lower in patients with ischemic heart disease accompanied by heart failure [25]. Furlani et al. [26] described an experimental canine model of myocardial infarction in which an induction of therapeutic angiogenesis by intramuscular administration of VEGF resulted in the maintenance of left ventricular function. An increased number of capillary vessels within the myocardium was observed, especially in the transition region between the infarcted and normal myocardium, while the number of arterioles was significantly lower than in the control group [26].

Our study showed significantly higher levels of serum VEGF in patients with more advanced stages of heart failure. One of the latest studies evaluated the efficacy and clinical significance of administration of the VEGF encoding gene [27]. The authors reported a significant improvement in myocardial perfusion in patients who received VEGF in combination with adenoviral vector after 6 months. Enhanced VEGF concentration in patients from higher NYHA classes can be associated with its repair properties, and the absence of elevated concentration in patients with collaterals seems to be associated with greater capillary circulation area, rather than the formation of arterioles.

One of the most important functions of FGF is the stimulation of endothelial cell proliferation and organization of the formed cells into tubular structures to induce angiogenesis. FGF1 and FGF2 are more potent stimulators of angiogenesis than VEGF and PDGF. The efficacy and safety of FGF1 was evaluated in 40 patients with three-vessel disease undergoing CABG. Twenty patients in this group received an FGF1 injection near the left descending coronary artery anastomosis. Pecher et al. concluded that after three years, the network of blood vessels resulting from the application of FGF1 was clearly visible and the followed patients presented better exercise tolerance and increased left ventricular ejection fraction [28]. In our observations, FGFbH serum levels in all three blood samples were significantly higher than in the control group. Moreover, in patients with developed collaterals and heart failure, FGFbH concentration in the coronary arteries was higher, which may indicate not only a stimulating role of FGFbH in angiogenesis, but also in arteriogenesis.

A meta-analysis by Meier et al. showed that the presence of collaterals in patients with ischemic heart disease reduced mortality [29]. According to W. Schaper, who commented on the above meta-analysis, if the prognosis in patients with collaterals is better than with CABG, and maybe even stenting, then there is a chance for improved prognosis for the patients in whom heart failure progresses when other treatment options have been exhausted [30].

Limitations

The main limitation is the small size of our group of patients. Additionally, we cannot exclude the effects of drug therapy on our results. Our findings help to advance knowledge on this topic and are of potential practical value.

The authors concluded that higher levels of pro-inflammatory factor IL-6 and growth factor FGFbH were observed in patients with heart failure, especially in the more advanced stages of HF. The concentrations of pro-inflammatory factors, growth factors and endostatin were significantly associated with the presence of coronary collaterals; however, a direct relationship was demonstrated only for higher levels of FGFbH and collaterals formation. Collaterals formation is associated with the activation of pro-inflammatory factors, growth factors and endostatin.

Acknowledgements. The authors thank "Proper Medical Writing" for the language assistance provided in the preparation of this paper.

References

[1] **Al Haj Zen A, Madeddu P:** Notch signalling in ischemia-induced angiogenesis. Biochem Soc Trans 2009, 37, 1221–1229.

[2] **Heilmann C, Beyersdorf F, Lutter G:** Collateral growth: cells arrive at the construction site. Cardiovasc Surg 2002, 10, 570–578.

[3] **Emanueli C, Madeddu P:** Therapeutic angiogenesis: translating experimental concepts to medically relevant goals. Vasc Pharmacol 2006, 45, 334–339.

[4] **Pipp F, Boehm S, Cai WJ, Adili F, Ziegler B, Karanovic G, Ritter R, Balzer J, Scheler C, Schaper W, Schmitz--Rixen T:** Elevated fluid shear stress enhances postocclusive collateral artery growth and gene expression in the pig hind limb. Arterioscler Thromb Vasc Biol 2004, 24, 1664–1668.

[5] **Schierling W, Troidl K, Troidl C, Schmitz-Rixen T, Schaper W, Eitenmüller IK:** The role of angiogenic growth factors in arteriogenesis. J Vasc Res 2009, 46, 365–374.

[6] **Buschmann I, Heil M, Jost M, Schaper W:** Influence of inflammatory cytokines on arteriogenesis. Microcirculation 2003, 10, 371–379.

[7] **Griffioen AW, Molema G:** Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. Pharmacol Rev 2000, 52, 237–268.

[8] **Tabibiazar R, Rockson SG:** Angiogenesis and the ischaemic heart. Eur Heart J 2001, 22, 903–918.

[9] **Chung NA, Lydakis C, Belgore F:** Angiogenesis in myocardial infarction. An acute or chronic process? Eur Heart J 2002, 23, 1604–1608.

[10] **Panchal VR, Rehman J, Nguyen AT, Brown JW, Turrentine MW, Mahomed Y, March KL:** Reduced pericardial levels of endostatin correlate with collateral development in patients with ischemic heart disease. J Am Coll Cardiol 2004, 43, 1383–1387.

[11] **Charney R, Cohen M:** The role of the coronary collateral circulation in limiting myocardial ischemia and infarct size. Am Heart J 1993, 126, 937–945.

[12] **Lahteenvuo JE, Lahteenvuo MT, Kivela A, Rosenlew C, Falkevall A, Klar J, Heikura T, Rissanen TT, Vahakangas E, Korpisalo P, Enholm B, Carmeliet P, Alitalo K, El-Melegy NT, Mohamed NA:** Angiogenic biomarkers in children with congenital heart disease: possible implications. Ital J Pediatr 2010, 36, 32.

[13] **Cohen M, Rentrop KP:** Limitation of myocardial ischaemia by collateral circulation during sudden controlled coronary artery occlusion in human subjects. A prospective study. Circulation 1974, 469, 1986–1992.

[14] **Rentrop KP, Feit F, Sherman W, Thornton JC:** Serial angiographic assessment of coronary artery obstruction and collateral flow in acute myocardial infarction. Report from the second Mount Sinai-New York University Reperfusion Trial. Circulation 1989, 80, 1166–1175.

[15] **Pipp F, Boehm S, Cai WJ, Adili F, Ziegler B, Karanovic G, Ritter R, Balzer J, Scheler C, Schaper W, Schmitz--Rixen T:** Elevated fluid shear stress enhances postocclusive collateral artery growth and gene expression in the pig hind limb. Arterioscler Thromb Vasc Biol 2004, 24, 1664–1668.

[16] **Baumann H, Gauldie J:** The acute phase response. Immunol Today 1994, 15, 74–80.

[17] **Weyand CM, Hicok KC, Hunder GG, Goronzy JJ:** Tissue cytokine patterns in patients with polymyalgia rheumatica and giant-cell arteritis. Ann Intern Med 1994, 121, 484–491.

[18] **Hernández-Rodríguez J, Segarra M, Vilardell C, Sánchez M, García-Martínez A, Esteban MJ, Queralt C, Grau JM, Urbano-Márquez A, Palacín A, Colomer D, Cid MC:** Tissue production of proinflammatory cytokines (IL-1ß, TNF-{alpha}, and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. Rheumatology 2004, 43, 294–301.

[19] **Mantovani A, Garlanda C, Introna M, Vecchi A:** Regulation of endothelial cell function by pro- and anti-inflammatory cytokines. Transplant Proc 1998, 30, 4239–4243.

[20] **Rossi V, Breviario F, Ghezzi P, Dejana E, Mantovani A:** Prostacyclin synthesis induced by in vascular cells by interleukin-1. Science 1985, 225, 174–176.

[21] **Aoki Y, Jaffe ES, Chang Y, Jones K, Teruya-Feldstein J, Moore PS, Tosato G:** Angiogenesis and hematopoiesis induced by Kaposi-sarcoma-associated herpes-virus encoded interleukin-6. Blood 1999, 93, 4034–4043.

[22] **Zhong Z, Wen Z, Darnell JE Jr:** Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994, 264, 95–98.

[23] **Valdembri D, Serini G, Vacca A, Ribatti D, Bussolino F:** *In vivo* activation of JAK2/STAT-3 pathway during angiogenesis induced by GM-CSF. FASEB J 2002, 16, 225–227.

[24] **El-Melegy NT, Mohamed NA:** Angiogenic biomarkers in children with congenital heart disease: possible implications. Ital J Pediatr 2010, 36, 32.

[25] **Patel JV, Abraheem A, Chackathayil J, Gunning M, Creamer J, Hughes EA, Lip GY:** Circulating biomarkers of angiogenesis as indicators of left ventricular systolic dysfunction amongst patients with coronary artery disease. J Intern Med 2009, 265, 562–567.

[26] **Furlani AP, Kalil RA, Castro I, Canedo-Delgado A, Barra M, Prates PR, Sant`Anna RT, Nesralla IA:** Effects of therapeutic angiogenesis with plasmid VEGF165 on ventricular function in a canine model of chronic myocardial infarction. Rev Bras Cir Cardiovasc 2009, 24, 143–149.

[27] **Hedman M, Muona K, Hedman A, Kivelä A, Syvänne M, Eränen J, Rantala A, Stjernvall J, Nieminen MS, Hartikainen J, Ylä-Herttuala S:** Eight-year safety follow-up of coronary artery disease patients after local intracoronary VEGF gene transfer. Gene Ther 2009, 16, 629–634.

[28] Pecher P, Schumacher BA: Angiogenesis in ischemic human myocardium: clinical results after 3 years. Ann Thorac Surg 2000, 69, 1414–1419.
[29] Meier P, Hemingway H, Lansky AJ, Knapp G, Pitt B, Seiler Ch: The impact of the coronary collateral circulation on mortality: a meta-analysis. Eur Heart J 2012, 33, 614–621.
[30] Schaper W: Collateral vessels reduce mortality. Eur Heart J 2012, 33, 564–566.

Address for correspondence:

Małgorzata Kobusiak-Prokopowicz
Department and Clinic of Cardiology
Wroclaw Medical University
Borowska 213
50-514 Wrocław
Poland
Tel.: +48 71 376 42 00
E-mail: kobusiak@poczta.fm

Conflict of interest: None declared

Received: 12.11.2014
Revised: 22.01.2015
Accepted: 23.02.2015