

Associations between selected angiographic parameters and the number of CD34⁺ cells and plasma levels of vascular endothelial growth factor and angiogenin in patients with ST-segment elevation myocardial infarction

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KEY WORDS

angiogenic factors,
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ABSTRACT

INTRODUCTION Left ventricular (LV) function and prognosis in patients after myocardial infarction are associated with some angiographic parameters.

OBJECTIVES The aim of the study was to assess the associations between the TIMI score in the infarct-related artery (IRA) before percutaneous coronary intervention (PCI), myocardial blush grade (MBG) following effective PCI, and the extent of collaterals measured using the Rentrop scale and plasma levels of vascular endothelial growth factor (VEGF) and angiogenin, number of CD34⁺ cells, as well as LV ejection fraction (LVEF) and wall motion score index (WMSI).

PATIENTS AND METHODS In 62 patients with the first ST-segment elevation myocardial infarction (STEMI) treated with PCI and bare metal stent implantation, plasma VEGF and angiogenin levels as well as the number of CD34⁺ cells were assessed before PCI, 24 hours after PCI, at discharge, and at 30 days following STEMI. LVEF and WMSI were evaluated by echocardiography at discharge and at 1 and 6 months after STEMI.

RESULTS Patients with TIMI 0–1 flow in the IRA before PCI (64.6% of the patients) had significantly higher troponin I and VEGF levels as well as a higher number of CD34⁺ cells than patients with TIMI 3 flow. Patients with TIMI 0–1 flow also had worse LV systolic function at 1 and 6 months following STEMI. Neither the MBG grade nor the Rentrop score showed associations with the mobilization of CD34⁺ cells, VEGF and angiogenin levels, and parameters of LV systolic function.

CONCLUSIONS Early patency of the IRA and lower myocardial necrosis seem to be more important for LV function assessed in patients 6 months after STEMI than mobilization of CD34⁺ cells and levels of angiogenic factors.

INTRODUCTION Over the past 15 years, the processes of heart regeneration have been extensively studied. It is now well known that the adult heart has a potential for natural regeneration and approximately half of the heart mass will be renewed over an individual's life.^{1–3} It is also known that

cardiomyocyte renewal intensifies in a pathological condition such as acute myocardial infarction (MI). Within 5 to 7 days after the acute phase of MI, reparative processes are initiated.² They include scar formation, cardiomyocyte regeneration, and neoangiogenesis. There are numerous

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factors engaged in this process, such as progenitor cells, proangiogenic factors, cytokines, kinins, chemokines, and bioactive phospholipids.^{1,2} The extension of postinfarction heart regeneration is related to age, sex, risk factors, physical activity, and severity of coronary artery disease.^{1,4} Numerous studies confirmed an increased number of progenitor cells in peripheral blood in patients with ST-segment elevation MI (STEMI). The mechanism by which progenitor cells act and contribute to heart regeneration is unclear. Currently, it is believed that paracrine mechanisms accompanying stem cell mobilization play a significant role in postinfarction regeneration of the heart.^{2,3,5} Paracrine effects include inhibition of apoptosis, protection against ischemic damage of cardiomyocytes, and induction of angiogenesis. The extent of myocardial necrosis after acute ischemia is influenced by many factors. The most important include the duration of ischemia, patency of the infarct-related artery (IRA), normal myocardial perfusion following effective coronary angioplasty, and the presence or absence of collateral circulation. These factors also affect prognosis in patients after MI.^{6,7}

The aim of this study was to examine relationships between some angiographic parameters and the level of angiogenic factors involved in postinfarction regeneration of the myocardium and neoangiogenesis, such as vascular endothelial growth factor (VEGF), angiogenin, and CD34⁺ progenitor cells in patients with the first MI treated with primary coronary angioplasty. Moreover, relationships between the above angiographic parameters and angiogenic factors on the one hand and left ventricular ejection fraction (LVEF) and the wall motion score index (WMSI) evaluated by echocardiography on the other, were also assessed. Similar investigations were performed in the past but the results were not referred to thrombolysis in myocardial infarction (TIMI) flow before the intervention. Moreover, angiogenin was not evaluated in this context.

PATIENTS AND METHODS The study group comprised 62 patients with the first STEMI. Patients with acute cardiac failure secondary to MI, with mechanical complications, hepatic or renal insufficiency or those presenting symptoms of current infection were excluded from the study. The study was approved by the Ethics Committee for Scientific Research (BW 40/2006). All patients gave their written consent to participate in the study.

All patients were treated with primary percutaneous coronary intervention (pPCI) combined with bare metal stent (BMS) implantation. Coronary angiography was evaluated post hoc by 2 independent interventional cardiologists. The following parameters were evaluated: 1) flow in the IRA before and after PCI with the TIMI scoring system (grades from 0 to 3); 2) myocardial perfusion following effective pPCI defined as the myocardial blush grade (MBG) (grades from 0 to 3); and 3) the extent of collateral development

measured using the Rentrop score (grades from 0 to 3). The choice of these scores was dictated by their common recognition and well-documented clinical usefulness.⁸⁻¹⁰

Blood samples were taken immediately before angioplasty, and then at 6 and 24 hours after PCI, at hospital discharge (day 5 of hospitalization for uncomplicated inferior wall infarction and day 7 for anterior wall infarction), and, finally, 30 days after STEMI. Blood for troponin I (TnI) measurement was drawn directly before pPCI and 6 hours after the procedure, including only the latter time point in a statistical analysis. Blood samples taken before pPCI, at 24 hours, and later were centrifuged (3000 rpm/15 min) and the obtained plasma was frozen to -70°C. Plasma levels of VEGF and angiogenin were measured using BD FACS Array Bioanalyzer System (Becton Dickinson, San Jose, California, United States). For complete blood count as well as the evaluation of the number and proportion of CD34⁺ cells, EDTA tubes were used. The analysis was conducted using a Coulter Cytomix FC-500 cytometer. Peripheral blood samples with EDTA were labelled with monoclonal anti-CD34⁺ antibodies according to the manufacturer's instructions with simultaneous erythrocyte lysis using the UtiLyse reagent (DAKO, Glostrup, Denmark). Appropriate isotypic G immunoglobulins were used as a negative control. The analysis was performed each time on approximately 30 000 leukocytes. The number of cells with positive antigen expression in the lymphocyte gate was measured, and their proportion in relation to all peripheral blood leukocytes was determined. The tests were performed 3 times at time points consistent with the measurement of angiogenic factors.

Echocardiographic examination with a SONOS 7500 machine was carried out at discharge (between days 5 and 7), at 30 days, and 6 months following STEMI and was performed by the same 2 echocardiographers throughout the entire study. LVEF was assessed with the Teichholz method, and the WMSI was calculated. In order to evaluate segmental motion disorders, standard division of the left ventricular (LV) muscle into 17 segments was applied. Wall motion was classified as normokinesis, hypokinesis, akinesis, and dyskinesis, and scores from 1 to 4, respectively, were assigned for each segment.

During 6 months of follow-up, all patients received aspirin, clopidogrel, angiotensin-converting enzyme inhibitor (ramipril or perindopril), β -blocker (bisoprolol or metoprolol succinate), and statin (simvastatin or atorvastatin). The statistical analysis was performed using the STATISTICA version 7.1. software (StatSoft®, Kraków, Poland). Normally distributed variables were reported as mean values with standard deviation, and nonnormally distributed variables as medians and interquartile range. Statistical significance of related variables was verified with the Friedman and Wilcoxon tests, while for unrelated variables, the Kruskal-Wallis and Mann-Whitney

TABLE 1 Characteristics of the study group (n = 62)

Parameter	Value
mean age, y	59.8 ± 10.1
sex, male	48 (77.4)
diabetes mellitus	20 (32.3)
smoking	31 (50)
hypertension	21 (33.8)
mean time from the onset of chest pain to the time of coronary intervention, min	278 ± 191
patients with anterior wall MI	30 (48.3)
patients with inferior wall MI	32 (51.7)
patients with 1-vessel disease	30 (48.5)
patients with 2-vessel disease	20 (32.2)
patients with 3-vessel disease	12 (19.3)
TIMI 0–1 flow in IRA before PCI	40 (64.6)
TIMI 2 flow in IRA before PCI	10 (16.1)
TIMI 3 flow in IRA after PCI	12 (16.3)
MBG 0–1 after PCI	8 (13)
MBG 2 after PCI	27 (43.5)
MBG 3 after PCI	27 (43.5)

Data are presented as number (percentage) or mean ± standard deviation.

Abbreviations: IRA, infarct-related artery; MBG, myocardial blush grade; MI, myocardial infarction; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction

tests were used. A *P* value of 0.05 or lower was considered significant. The calculated Spearman's correlation coefficient values were also considered significant at a *P* value of 0.05 or lower.

RESULTS The characteristics of the study group are presented in **TABLE 1**. Of the 62 patients with STEMI, 30 patients (48.3%) had anterior wall MI, and 32 patients (51.7%) had inferior wall MI. The mean pain-to-balloon time was 278 ± 191 minutes. Patients with anterior wall MI had significantly higher peak TnI levels (measured 6 hours after PCI) compared with patients with inferior wall MI (43.3 ± 13.7 vs 29.4 ± 19.3 ng/ml; *P* < 0.001). No significant differences were observed in the number of CD34⁺ progenitor cells and the range of angiogenic factor levels with regard to diabetes, arterial hypertension, and smoking status.

TIMI 0 and TIMI 1 flow in the IRA before PCI was found in 36 and 4 patients, respectively. These patients constituted group 1 (64.6% of the study group). Twelve patients (19.3%) had patent IRA with TIMI 3 flow, while in the remaining 10 patients (16.1%), TIMI 2 flow was identified. Following coronary intervention, TIMI 3 flow was found in 60 patients (96.7%), and MBG 3 (normal) and MBG 2 (slightly impaired) were observed in 27 patients each (43.5%). To simplify statistical calculations, the only patient with MBG 0 was reported in the MBG-1 group (8 patients). In 66% of the patients (40 patients), a total absence of collateral circulation was observed (Rentrop

0 score). Rentrop scores 1 and 2 were identified in 11 patients each. There were no patients with Rentrop score 3.

TIMI flow and tested parameters Patients with an occluded IRA during coronary angiography (TIMI 0–1 flow), had the highest TnI levels, showing significant differences when compared with patients with TIMI 3 flow. The measurement performed before PCI showed significantly higher CD34⁺ cell counts in the TIMI-0–1 group as compared with TIMI-3 patients (161.7 ± 74.8 vs 29.5 ± 25.5/μl; *P* = 0.02). At subsequent measurement time points, the number of CD34⁺ cells was lower and unrelated to TIMI flow in the IRA before PCI. VEGF levels before PCI in TIMI-0–1 patients were higher than in the TIMI-2 or TIMI-3 group, with the differences consistently present at each measurement time point, although not significant. Angiogenin levels showed no significant differences regarding the TIMI flow grade in the IRA before PCI (**TABLE 2**).

Myocardial blush grade and the number of CD34⁺ cells and tested angiogenic factors The number of CD34⁺ cells was the highest in the MBG-0–1 group at each time point, but the difference between MBG-2 and MBG-3 patients was not significant (**TABLE 3**). In MBG-0–1 patients, VEGF levels measured before PCI, at discharge, and 30 days following STEMI were higher than in the MBG-2 and MBG-3 groups, but the differences were not significant. VEGF levels measured 2 days after STEMI were the highest in the MBG-3 group, and the differences compared with MBG-0 patients showed only borderline significance (1681.1 ± 3349.5 vs 472.3 ± 435.4; *P* = 0.053). Angiogenin levels showed no relation with MBG. TnI levels measured 6 hours following PCI, although the highest in MBG-0–1 patients (44.4 ± 11.4 ng/ml), revealed no significant difference in comparison with patients with MBG 2 (30.1 ± 19.6 ng/ml) and MBG 3 (33.3 ± 18.6 ng/ml). The extent of collateral circulation development evaluated by the Rentrop score showed no relationship with any of the studied factors (**TABLE 4**).

Angiographic parameters and left ventricular systolic function TIMI flow in the IRA before PCI correlated with both LVEF measured at 1 and 6 months after MI as well as WMSI measured at all time points: LVEF was significantly higher and WMSI was significantly lower with TIMI 3 flows than with TIMI 0–1 flow (**TABLE 5**). Neither MBG nor the extent of collateral circulation showed correlations with the parameters of LV systolic function at any of the time points.

Correlations None of the angiogenic factors showed correlations with TnI levels, LVEF, and WMSI. A negative correlation between the number of CD34⁺ cells measured at discharge and WMSI assessed 6 months after STEMI was found (*r*² = −0.31; *P* < 0.05).

TABLE 2 Levels of troponin I (measured 6 hours after percutaneous coronary intervention), vascular endothelial growth factor, angiogenin, and CD34⁺ cell count in relation to TIMI flow in infarct-related artery before the treatment with primary coronary angioplasty in patients with ST-segment elevation myocardial infarction

Parameter	n	TIMI 0–1	n	TIMI 2	n	TIMI 3	P value
troponin I, ng/ml	40	42.40 ± 13.87	10	26.46 ± 19.36	12	15.11 ± 12.9	0.0004 ^{0 vs 3}
CD34 ⁺ (1), count/μl	40	161.7 ± 74.8	10	30.55 ± 21.5	12	29.5 ± 5.5	0.02 ^{0 vs 3}
CD34 ⁺ (2), count/μl	39	124 ± 45.1	9	59.7 ± 16.22	11	35.0 ± 34.0	NS
CD34 ⁺ (3), count/μl	35	42.2 ± 8.7	10	24.3 ± 13.2	8	32.0 ± 22.4	NS
VEGF (1), pg/ml	40	1565.7 ± 3598.9	8	987.7 ± 542.3	11	854.46 ± 576.34	NS
VEGF (2), pg/ml	35	1076.5 ± 2587.9	8	726.2 ± 542.2	10	721.5 ± 634.4	NS
VEGF (3), pg/ml	38	1167.6 ± 1607.8	8	647.4 ± 798.6	11	760.4 ± 418.3	NS
VEGF (4), pg/ml	38	1399.9 ± 2673.9	7	593.0 ± 743.9	10	735.1 ± 601.2	NS
angiogenin (1), ng/ml	40	306511.4 ± 357819.5	9	198446.7 ± 49996.3	11	488212.6 ± 857669.9	NS
angiogenin (2), ng/ml	38	222264.3 ± 84804.3	9	193731.8 ± 61608.4	10	182930.4 ± 77506.6	NS
angiogenin (3), ng/ml	40	227243.5 ± 117409.5	9	187666.9 ± 47306.9	11	244686.1 ± 135041.2	NS
angiogenin (4), ng/ml	40	594048.7 ± 236137.2	9	175830.7 ± 55523.6	9	745436.7 ± 168687.1	NS

Data are presented as mean ± standard deviation.

The numbers in brackets refer to the time of measurements: 1, before PCI; 2, 24 hours after PCI; 3, on the day of discharge; 4, 30 days after myocardial infarction

Abbreviations: NS, not significant; VEGF, vascular endothelial growth factor; others, see [TABLE 1](#)

TABLE 3 Levels of troponin I (measured 6 hours after percutaneous coronary intervention), vascular endothelial growth factor, angiogenin, and CD34⁺ cell count in relation to myocardial blush grade assessed after percutaneous coronary intervention in patients with ST-segment elevation myocardial infarction

Parameter	n	MBG 0–1	n	MBG 2	n	MBG 3	P value
troponin I, ng/ml	8	44.4 ± 11.4	27	30.1 ± 19.6	27	33.3 ± 18.6	NS
CD34 ⁺ (1), count/μl	8	38.6 ± 11.6	27	17.4 ± 14.3	27	23.3 ± 21.9	NS
CD34 ⁺ (2), count/μl	8	276.0 ± 68.9	25	297.7 ± 111.2	25	21.9 ± 25.4	NS
CD34 ⁺ (3), count/μl	8	72.1 ± 12.8	22	21.6 ± 15.4	24	26.3 ± 16.1	NS
VEGF (1), pg/ml	8	1864.2 ± 4478.2	27	1519.9 ± 3503.9	27	892.7 ± 626.1	NS
VEGF (2), pg/ml	7	472.3 ± 435.4	22	490.9 ± 289.3	25	1681.1 ± 3349.5	0.053 ^{1 vs 3}
VEGF (3), pg/ml	8	1411.0 ± 2163.1	25	823.2 ± 896.7	23	951.1 ± 1120.5	NS
VEGF (4), pg/ml	7	2485.7 ± 4158.8	23	528.5 ± 342.8	24	906.3 ± 937.7	0.055 ^{1 vs 2}
angiogenin (1), ng/ml	8	298037.2 ± 296446.1	27	304721.2 ± 435087.9	27	332212.2 ± 595049.8	NS
angiogenin (2), ng/ml	7	226434.7 ± 95502.6	22	200582.4 ± 72175.6	25	212831.4 ± 83921.9	NS
angiogenin (3), ng/ml	8	249153.9 ± 157379.0	25	223393.2 ± 104562.3	23	212027.5 ± 95696.3	NS
angiogenin (4), ng/ml	7	183234.0 ± 126792.0	25	180813.0 ± 52851.0	23	122197.8 ± 34107.0	NS

Data are presented as mean ± standard deviation.

The numbers in brackets refer to the time of measurements: 1, before PCI; 2, 24 hours after PCI; 3, on the day of discharge; 4, 30 days after myocardial infarction

Abbreviations: see [TABLES 1 and 2](#)

DISCUSSION Acute myocardial ischemia, as a consequence of IRA occlusion, leads to cardiomyocyte necrosis, induces apoptosis, and activates natural repair mechanisms. Irrespective of the size of the necrotic area, maladaptive heart remodeling is initiated.^{11,12} Early and effective restoration of blood flow in the IRA and normalization of myocardial perfusion are well known factors limiting the extension of necrosis and thus improving LV function and prognosis after MI.^{13–19} Well-developed collateral circulation should also be expected to protect against ischemic heart injury, but the results of studies are

inconsistent. Acute myocardial ischemia initiates a systemic inflammatory response that induces a signaling cascade.^{20–23} One of the released factors is hypoxia-induced factor 1, which stimulates the secretion of growth factors such as VEGF and fibroblast growth factor.^{11,24} VEGF contributes to neoangiogenesis and reendothelialization of the damaged vessels. Fibroblast growth factor, although its levels rise in acute MI, did not show the expected effect stimulating the development of collaterals in previous studies and did not prove to be a prognostic factor.^{11,24} In one of few studies evaluating angiogenin activity in

TABLE 4 Levels of troponin I (measured 6 hours after percutaneous coronary intervention), vascular endothelial growth factor, angiogenin, and CD34⁺ cell count in relation to the Rentrop score in patients with ST-elevation myocardial infarction

Parameter	n	Rentrop 0	n	Rentrop 1	n	Rentrop 2	P value
troponin I, ng/ml	8	44.4 ± 11.4	27	30.1 ± 19.6	27	33.3 ± 18.6	NS
CD34 ⁺ (1), count/μl	8	38.6 ± 11.6	27	17.4 ± 14.3	27	23.3 ± 21.9	NS
CD34 ⁺ (2), count/μl	8	276.0 ± 68.9	25	297.7 ± 111.2	25	21.9 ± 25.4	NS
CD34 ⁺ (3), count/μl	8	72.1 ± 12.8	22	21.6 ± 15.4	24	26.3 ± 16.1	NS
VEGF (1), pg/ml	8	1864.2 ± 4478.2	27	1519.9 ± 3503.9	27	892.7 ± 626.1	NS
VEGF (2), pg/ml	7	472.3 ± 435.4	22	490.9 ± 289.3	25	1681.1 ± 3349.5	0.053 ^{1 vs 3}
VEGF (3), pg/ml	8	1411.0 ± 2163.1	25	823.2 ± 896.7	23	951.1 ± 1120.5	NS
VEGF (4), pg/ml	7	2485.7 ± 4158.8	23	528.5 ± 342.8	24	906.3 ± 937.7	0.055 ^{1 vs 2}
angiogenin (1), ng/ml	8	298037.2 ± 296446.1	27	304721.2 ± 435087.9	27	332212.2 ± 595049.8	NS
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Data are presented as mean ± standard deviation.

The numbers in brackets refer to the time of measurements: 1, before PCI; 2, 24 hours after PCI; 3, on the day of discharge; 4, 30 days after myocardial infarction

Abbreviations: see TABLES 1 and 2

TABLE 5 Associations between TIMI flow in infarct-related artery before primary coronary angioplasty and parameters assessing left ventricular systolic function examined at discharge (1), 30 days (2), and 6 months (3) after ST-segment elevation myocardial infarction

Parameter	n	TIMI 0–1	n	TIMI 2	n	TIMI 3	P value
LVEF (1), %	40	43.2 ± 6.5	10	43.4 ± 6.1	12	48.4 ± 5.92	NS
LVEF (2), %	40	44.6 ± 6.8	10	44.6 ± 8.2	12	51.6.0 ± 3.1	0.01 ^{1 vs 3}
LVEF (3), %	39	44.6 ± 8.8	9	47.5 ± 6.6	9	52.0 ± 5.8	0.02 ^{0 vs 3}
WMSI (1)	40	1.63 ± 0.21	10	1.59 ± 0.23	12	1.44 ± 0.16	0.03 ^{0 vs 3}
WMSI (2)	40	1.6 ± 0.2	10	1.53 ± 0.2	12	1.35 ± 0.09	0.009 ^{0 vs 3}
WMSI (3)	39	1.58 ± 0.24	9	1.49 ± 0.26	9	1.32 ± 0.08	0.01 ^{0 vs 3}

Data are presented as means ± standard deviation.

Abbreviations: LVEF, left ventricular ejection fraction; WMSI, wall motion score index; others, see TABLES 1 and 2

the setting of acute MI, increased levels of angiogenin were associated with worse prognosis in a 6-month follow-up.^{25,26} The cells that participate in heart regeneration also include progenitor cells mobilized from bone marrow and from organ niche by numerous chemoattractants, for example, cytokines, growth factors, kinins, and bioactive phospholipids.^{2,5} Among many others, their surface markers include the CD34 antigen.

In our study, we examined the effect of TIMI flow in the IRA before PCI, MBG following PCI, and the extent of collateral circulation development on the number of CD34⁺ cells and serum concentrations of factors that affect their mobilization and contribute to protecting the heart against infarction-triggered tissue destruction. In some cases of MI, a patent IRA with TIMI 3 flow can be observed on angiography before PCI. The incidence of this finding in our study was 19.3% and was similar to that reported by other authors.¹³ Preserved blood flow in the IRA (due to its spontaneous recanalization or its incomplete occlusion) should limit the process of myocardial

necrosis and inhibit the stimulus that induces secretion of growth factors. In a study comparing MI patients with TIMI 3 flow in the IRA before PCI with TIMI-0–2 patients, it was found that those with TIMI 3 flow had a more favorable prognosis owing to better LV function.¹³

In our study, TnI levels, the number of CD34⁺ precursor cells on admission to the hospital, and VEGF levels at each measurement time point were higher in patients with TIMI 0–1 flow in the IRA before PCI as compared with TIMI-3 patients (findings most likely to be attributed to more extensive necrosis and regeneration activity in the former group). Despite this, patients with TIMI 0–1 flow in the IRA before PCI presented with significantly lower LVEF and a significantly higher WMSI at 1 and 6 months after MI, compared with TIMI-3 flow patients. This suggests that patients with MI, in whom rapid spontaneous recanalization or incomplete occlusion of the IRA occurs, have a better LV systolic function owing to limited myocardial necrosis rather than enhanced myocardial regeneration. Our results are somewhat

different to those reported by Grøgaard et al.,²⁷ who found that the number of CD34⁺ cells increased in MI patients just after PCI, but they did not assess the number of CD34⁺ before PCI. It is possible that if such an assessment had been done, they could have found a higher number of CD34⁺ cells before PCI than they did after the procedure. In their study, the levels of some growth factors were also measured, featuring stromal-derived 1 α factor (SDF-1 α), hepatocyte growth factor (HGF), and insulin-like growth factor 1 (IGF-1). The highest levels of SDF-1 α and HGF were found 14 days after MI and 3 hours after PCI, respectively. The level of IGF-1 remained unchanged. The authors suggested that the highest level of SDF-1 α indicates the most suitable time and environment for the initiation of heart regeneration.²⁷ This study also showed a significant negative correlation between TIMI perfusion grade and the number of CD34⁺ cells measured 5 days after MI.

Microcirculation perfusion is a better indicator of the effectiveness of PCI than TIMI flow in the IRA.^{15-18,28-30} In our study, TIMI 3 flow grade in the IRA following PCI was found in 96.7% of the patients, but myocardial perfusion evaluated as MBG 3 grade was observed only in 43.5% of the patients. We expected that patients with lower MBG grades would have more extensive myocardial necrosis and worse LV systolic function parameters. However, we found no significant differences either in TnI levels or in LVEF and WMSI between patients with MBG 0 and those with MBG 3. Also, the higher number of CD34⁺ cells and higher VEGF levels seen in MBG-0 patients compared with those with MBG 1–3 did not translate into significant differences regarding LVEF and WMSI. These discrepancies may be due to the applied method of evaluation of microcirculatory perfusion, namely, MBG. Visual assessment of myocardial perfusion, such as MBG, is highly subjective and the true incidence of microcirculatory perfusion impairment is likely to be higher than that evaluated by the MBG grade. In a study using a magnetic resonance-based assessment of microcirculatory perfusion, it was found that microvascular obstruction occurs in 94% of the patients after MI treated with PCI, despite the patency of the IRA.²⁹

The presence of well-developed collaterals at the development of IRA occlusion should serve as a protective mechanism against myocardial necrosis. Several studies have confirmed this hypothesis but the results are inconsistent.³¹⁻³³ There are studies showing that the extent of collateral development has a beneficial effect on prognosis in STEMI only when PCI was performed with a delay of more than 4 hours from pain onset.³⁴ In our study, no relationship between the extent of collateral circulation development and the concentration of myocardial necrosis markers was found. Rechciński et al.³⁴ obtained similar results in their study on 330 patients with STEMI treated with PCI. They did not find any correlations

between collateral development assessed by the Rentrop score and infarct size assessed as LVEF and as a maximal activity of creatine kinase. They observed a significant negative correlation between TIMI flow in the IRA and the extent of collateral circulation.³⁴ Also, in our study, no correlations between the extent of collateral circulation and LVEF and WMSI at any of the measurement time points were present. This could possibly be due to the fact that the study group was small and collateral circulation did not develop in two-thirds of the patients. Collateral development depends on duration of ischemic heart disease, among other things. The poor development of collaterals seen in our patients could result from short-lasting coronary artery disease. Our patients were hospitalized with the first MI, most of them had no prior history of angina and nearly half of them (48.5%) had 1-vessel disease. We also did not find any relationship between the Rentrop score on the one hand and the number of CD34⁺ cells and the levels of VEGF and angiogenin on the other.

There are few studies exploring connections between the Rentrop score and growth factors in patients with STEMI. One of them reports that patients with acute MI who presented with a higher Rentrop score (2 or 3) rarely were diabetic or had a history of stable angina.³⁵ They also had lower LVEF after MI and showed a trend toward longer pain-to-balloon time. In this study, the Rentrop score of 2 or 3 was associated with a lower increase in angiopoietin-2 levels, but VEGF levels showed delayed reaction for acute ischemia compared with angiopoietin-2.³⁵

Although the role of stem cell mobilization observed in the setting of STEMI has not been fully explained, numerous studies demonstrated that greater mobilization of these cells was associated with better LV systolic function.³⁶ This became the basis for clinical studies in which stem cells were administered intracoronary. In some of these studies, an improvement in LVEF was seen in patients receiving treatment, in contrast to placebo groups.^{36,37} In our study, greater mobilization of the CD34⁺ cells was noted in patients with TIMI 0–1 flow than in those with TIMI 3 flow (although only the measurement before PCI showed statistical significance). On the other hand, the former group presented with a significantly lower LVEF and significantly higher WMSI at 1 and 6 months following MI. Thus, in this group of patients, greater mobilization of the CD34⁺ cells was not associated with better regeneration of the myocardium. However, we also observed a negative correlation between the number of CD34⁺ cells evaluated on the day of discharge and WMSI measured 6 months following MI. This observation is in line with the idea that the naturally induced mobilization of stem cells is too low to have a considerable influence on the improvement of LV systolic function. In the FIRSTLINE trial,³⁸ natural mobilization of stem cells was stimulated by administering granulocyte

colony stimulating factor (G-CSF) to patients after acute MI. A 20-fold increase in the number of CD34⁺ precursor cells in the circulation was observed compared with G-CSF-naïve patients. In the G-CSF group, an increase in LVEF was also observed from 48% ± 4% at baseline to 56% ± 9% 1 year after MI. In patients who did not receive G-CSF, LVEF was considerably lower.

In some studies, a correlation was demonstrated between CD34⁺ cell mobilization and VEGF levels.^{39,40} In our study, with VEGF levels evaluated on admission to the hospital, that is, at the moment when they reflected the induction of VEGF secretion by ischemia, we did not observe such a correlation. This could be due to the fact that VEGF levels measured on admission in our patients were modified by the administration of heparin bolus (5000 units), given to each patient in the ambulance. Heparin contributes to a rapid decrease in VEGF levels because of its thrombin-inhibiting effect (thrombin stimulates VEGF secretion) and it binds simultaneously to VEGF and to endothelial cells.^{39,41} It is believed that VEGF levels measured 5 to 7 days after MI reflect the activity of the neoangiogenesis process.^{39,40} In our study, VEGF levels measured on days 5–7 and 30 days after MI in patients with TIMI 0–1 flow in the IRA before PCI were higher than those in patients with TIMI 2 and TIMI 3 flow. This could suggest more intensive regeneration processes in the TIMI-0–1 group. Similar results were obtained in the CAPTURE study,¹¹ in which patients with TIMI 3 flow in an acute coronary syndrome-related artery were reported to have low VEGF levels (lower quartile) significantly more frequently than patients with TIMI flow of 1 and lower. However, the study failed to demonstrate a link between better 6-month prognosis and higher activity of the regeneration processes.^{11,40} Our findings are similar, namely, TIMI flow in the IRA before PCI affected LVEF and WMSI, but LVEF and WMSI showed no relationship with angiogenic factor levels. It is known that the fate of bone marrow progenitor cells mobilized during MI depends on the nature of the myocardial microenvironment.² This environment is probably different in cases when the IRA is occluded at the time of PCI, in contrast to when it is patent. We were unable to demonstrate such difference in the levels of VEGF and angiogenin.

Learning points Although TIMI 0–1 flow in the IRA before PCI is associated with more intensive mobilization of CD34⁺ cells and stronger activation of angiogenesis as reflected by higher VEGF levels at each measurement time point, LV systolic function in this group of patients remains worse than in individuals with patent IRA before PCI.

Study limitations Our study has a number of limitations. First, no data concerning the use of medications in our patients before MI are available. These data would be particularly useful for assessing the effect of some of the medications

(eg, statins) on CD34⁺ cell count.^{42,43} Second, owing to technical complications, some of the blood samples were drawn or stored improperly, and the obtained material was recognized as unusable and was withdrawn from the analysis. For that reason, at each measurement time point, there were patients in whom some measurements were missed.

Conclusions Our results suggest that it is the size of necrosis rather than subsequent myocardial regeneration that affects LV systolic function in patients after acute MI treated with primary PCI. For that reason, the restoration of IRA patency as early as possible remains the most important target in patients with STEMI.

Contribution statement ZG conceived the idea for the study and completed and analyzed the data. RD, AS, and IŚ contributed to the design of the study and were involved in data collection. KG and JK analyzed the data. All authors participated in manuscript edition and approved its final version.

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REFERENCES

- 1 Terzic A, Behfar A. Regenerative heart failure headed for optimization. *Eur Heart J*. 2014; 35: 1231–1234.
- 2 Klyachkin YM, Karapetyan AV, Ratajczak MZ, et al. The role of bioactive lipids in stem cells mobilization and homing: novel therapeutics for myocardial ischemia. *Biomed Res Int*. 2014; 2014: 653543.
- 3 Ratajczak MZ, Jadczyk T, Pędziwiatr D, et al. New advances in stem cell research: practical implications for regenerative medicine. *Pol Arch Med Wewn*. 2014; 124: 417–426.
- 4 Nasser BA, Ebel W, Dandel M, et al. Autologous CD133+ bone marrow cells and bypass grafting for regeneration of ischemic myocardium: the Cardio133 trial. *Eur Heart J*. 2014; 35: 1263–1274.
- 5 Krum H. Current clinical trials of cell-based Therapies in cardiac repair: too many variables spoil the stem cell broth. *Eur Heart J*. 2013; 35: 955–957.
- 6 Gibson C.M, Schömig A. Coronary and myocardial angiography. Angiographic assessment of both epicardial and myocardial perfusion. *Circulation*. 2004; 100: 3096–3105.
- 7 Mohri M, Tomoike H, Noma M, et al. Duration of ischemia is vital for collateral development: repeated brief coronary artery occlusion in conscious dogs. *Circ Res*. 1989; 64: 287–296.
- 8 Chesebro JH, Knatterud G, Roberts R, et al. Thrombolysis in Myocardial Infarction (TIMI) Trial, Phase I: a comparison between intravenous tissue plasminogen activator and intravenous streptokinase. Clinical findings through hospital discharge. *Circulation*. 1987; 76: 132–154.
- 9 Henriques JPS, Zijlstra F, van't Hof, et al. Angiographic assessment of reperfusion in acute myocardial infarction by myocardial blush grade. *Circulation*. 2003; 107: 2115–2119.
- 10 Rentrop KP, Feit F, Blanke H, et al. Serial angiographic assessment of coronary artery obstruction and collateral flow in acute myocardial infarction. *Circulation*. 1989; 80: 1166–1175.
- 11 Heeschen Ch, Dimmeler S, Hamm ChW, et al. on behalf of the CAPTURE (c7E3 Anti-platelet Therapy in Unstable Refractory angina) Investigators. Prognostic significance of angiogenic growth factor serum levels in patients with acute coronary syndromes. *Circulation*. 2003; 107: 524–530.
- 12 Mani K, Kotsis RN. Myocyte apoptosis: programming ventricular remodeling. *J Am Coll Cardiol*. 2003; 41: 761–764.
- 13 Stone GW, Cox D, Garcia E, et al. Normal flow (TIMI-3) before mechanical reperfusion therapy is an independent determinant of survival in acute myocardial infarction: analysis from Primary Angioplasty in Myocardial Infarction trials. *Circulation*. 2001; 104: 636–641.
- 14 Zeymer U, Schröder R, Neuhaus KI. [Patency, perfusion and prognosis in acute myocardial infarction]. *Herz*. 1999; 24: 421–429. German.
- 15 Bax M, De Winter RJ, Schotborgh CE, et al. Short- and long-term recovery of left ventricular function predicted at the time of primary percutaneous intervention in anterior myocardial infarction. *J Am Coll Cardiol*. 2004; 43: 534–541.

- 16 Sheiban I, Fragasso G, Rosano GMC, et al. Time course and determination of left ventricular function recovery after primary angioplasty in patients with acute myocardial infarction. *J Am Coll Cardiol.* 2001; 38: 464-471.
- 17 Hoffmann R, Haager P, Arning J, et al. Usefulness of myocardial blush grade early and late after primary coronary angioplasty for acute myocardial infarction in predicting left ventricular function. *Am J Cardiol.* 2003; 92: 105-119.
- 18 Porto I, Hamilton-Craig C, Brancati M, et al. Angiographic assessment of microvascular perfusion- myocardial blush in clinical practice. *Am Heart J.* 2010; 16: 1015-1022.
- 19 Stone GW, Peterson MA, Lansky AJ, et al. Impact of normalized myocardial perfusion after successful angioplasty in acute myocardial infarction. *J Am Coll Cardiol.* 2002; 39: 591-597.
- 20 Wojakowski W, Ratajczak M, Tendera M. Interleukin-8: more on the mechanisms of progenitor cells mobilization in acute coronary syndrome. *Eur Heart J.* 2006; 27: 1013-1015.
- 21 Schönig K, Busch G, Steppich B, et al. Interleukin-8 is associated with circulating CD133+ progenitor cells in acute myocardial infarction. *Eur Heart J.* 2006; 27: 1032-1037.
- 22 Karpiński Ł, Plaksej R, Derzho R, et al. Serum levels of interleukin-6, interleukin-10 and C-reactive protein in patients with myocardial infarction treated with primary angioplasty during 6-months follow up. *Pol Arch Med Wewn.* 2009; 119: 115-126.
- 23 Boehar N, Rapp J, Pandya S, et al. Rebuilding the damaged heart: the potential of cytokines and growth factors in the treatment of ischemic heart disease. *J Am Coll Cardiol.* 2010; 56: 1287-1297.
- 24 Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes. Part III: Biomarkers of oxidative stress and angiogenic growth factors. *Circulation.* 2006; 113: e289-e292.
- 25 Hu GF, Riordan JF. Angiogenin enhances actin acceleration of plasminogen activation. *Biochem Biophys Res Commun.* 1993; 197: 682-687.
- 26 Tello-Montoliu A, Marin E, Patel J, et al. Plasma angiogenin levels in acute coronary syndromes: implications for prognosis. *Eur Heart J.* 2007; 28: 3006-3011.
- 27 Grøgaard HK, Solheim S, Landverk KS, et al. Circulating CD34+ progenitor cells and growth factors in patients treated with percutaneous coronary intervention for acute myocardial infarction or stable angina pectoris. *Scand J Clin Lab Invest.* 2011; 71: 322-329.
- 28 Hoffmann R, Haager P, Lepper W, et al. Relation of coronary flow pattern to myocardial blush grade in patients with first acute myocardial infarction. *Heart.* 2003; 89: 1147-1151.
- 29 Jesel L, Morel O, Ohlmann P, et al. Role of pre-infarction angina and inflammatory status in the extent of microvascular obstruction detected by MRI in myocardial infarction patients treated by percutaneous coronary angioplasty. *Int J Cardiol.* 2007; 121: 139-147.
- 30 Streb W, Duszańska A, Stabryła-Deska J, et al. The comparison of contrast echocardiography and tissue Doppler imaging for evaluation of reperfused myocardium in patients with acute myocardial infarction. *Cardiol J.* 2008; 15: 548-554.
- 31 Habib GB, Heibig J, Forman SA, et al. Influence of coronary collateral vessels on myocardial infarct size in humans: results of phase 1 thrombolysis in myocardial infarction (TIMI) trial. The TIMI investigators. *Circulation.* 1999; 83: 739-746.
- 32 Eisman P, van't Hof AWJ, de Boer MJ, et al. Role of collateral circulation in the acute phase of ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention. *Eur Heart J.* 2004; 25: 854-858.
- 33 Nicolau JC, Nogueira PR, Pinto MA, et al. Early infarct artery collateral flow does not improve long-term survival following thrombolytic therapy for acute myocardial infarction. *Am J Cardiol.* 1999; 83: 21-26.
- 34 Rechciński T, Jasińska A, Peruga JZ, et al. Presence of coronary collaterals in ST- elevation myocardial infarction patients does not affect long-term outcome. *Pol Arch Med Wewn.* 2013; 123: 29-37.
- 35 Bo Huyn K, Young-Guk K, Ae-Young H, et al. Serial plasma levels of angiogenic factors in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Korean Circ J.* 2012; 42: 464-470.
- 36 Wojakowski W, Tendera M. Mobilization of bone marrow-derived progenitor cells in acute coronary syndromes. *Folia Histochem Cytobiol.* 2005; 43: 229-232.
- 37 Schaefer A, Meyer GP, Fuchs M, et al. Impact of intracoronary bone marrow cell transfer on diastolic function in patients after acute myocardial infarction: results from the BOOST trial. *Eur Heart J.* 2006; 27: 929-935.
- 38 Ince H, Petzsch M, Kleine HD, et al. Prevention of left ventricular remodeling with granulocyte colony stimulating factor after acute myocardial infarction. Final 1-year results of the Front-Integrated Revascularisation and Stem cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony Stimulating Factor (FIRSTLINE-AMI Trial). *Circulation.* 2005; 112: 173-180.
- 39 Dai J, Rabie ABM. VEGF: an essential mediator of both angiogenesis and endochondral ossification. *J Dent Res.* 2007; 86: 937-950.
- 40 Ogawa H, Suefui H, Soejima H, et al. Increased blood vascular endothelial growth factor levels in patients with acute myocardial infarction. *Cardiology.* 2000; 93: 93-99.
- 41 Pyda M, Korybalska K, Książek K, et al. Effect of heparin on blood vascular endothelial growth factor levels in patients with ST-segment elevation acute myocardial infarction undergoing primary percutaneous coronary intervention. *Am J Cardiol.* 2006; 98: 902-905.
- 42 Vasa M., Fichtischerer S, Adler K, et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation.* 2001; 103: 2885-2890.
- 43 Hibbert B, Ma X, Pourdjabbar A, et al. Pre-procedural atorvastatin mobilizes endothelial progenitor cells: clues to the salutary effects of statins on healing of stented human arteries. *PLoS One.* 2011; 6: e16413.

Ocena powiązań między wybranymi parametrami angiograficznymi a liczbą komórek CD34⁺ we krwi obwodowej oraz osoczowymi poziomami naczyniowego czynnika wzrostu śródbłónka i angiogeniny u pacjentów z zawałem serca z uniesieniem odcinka ST

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SŁOWA KLUCZOWE

czynniki angiogenne,
parametry
angiograficzne,
zawał serca

STRESZCZENIE

WPROWADZENIE Funkcja lewej komory (LK) i rokowanie u pacjentów po zawałe serca wiążą się z niektórymi parametrami angiograficznymi.

CELE Celem pracy była ocena powiązań między stopniem przepływu TIMI w tętnicy odpowiedzialnej za zawał (*infarct-related artery* – IRA) przed przeszłorną interwencją wieńcową (*percutaneous coronary intervention* – PCI), perfuzją mikrokrążenia po skutecznej PCI i rozwojem krążenia obocznego ocenianego za pomocą skali Rentropa, a poziomami osoczowymi naczyniowego czynnika wzrostu śródbłónka (*vascular endothelial growth factor* – VEGF) i angiogeniny, a także liczbą komórek CD34⁺, frakcją wyrzutową LK oraz wskaźnikiem kurczliwości (*wall motion score index* – WMSI).

PACJENCI I METODY U 62 pacjentów z pierwszym w życiu zawałem serca z uniesieniem odcinka ST (*ST-segment elevation myocardial infarction* – STEMI), których leczono za pomocą PCI z implantacją stentu metalowego, oznaczano poziomy VEGF i angiogeniny w osoczu oraz liczbę komórek CD34⁺ bezpośrednio przed PCI, 24 godziny po PCI, w dniu wypisu ze szpitala oraz 30 dni po STEMI. W dniu wypisu ze szpitala oraz miesiąc i 6 miesięcy po zawałe serca oceniono echokardiograficznie frakcję wyrzutową i wskaźnik kurczliwości LK.

WYNIKI U pacjentów z przepływem TIMI 0–1 w IRA przed PCI (64,6% badanej grupy) stwierdzono znamienne wyższe stężenia troponiny I i VEGF oraz większą liczbę komórek CD34⁺ niż u pacjentów z TIMI 3. Pacjenci z przepływem TIMI 0–1 mieli gorszą funkcję skurczową LK ocenianą miesiąc i 6 miesięcy po STEMI. Ani stopień perfuzji mikrokrążenia, ani stopień rozwoju krążenia obocznego nie wykazywały powiązań z mobilizacją komórek CD34⁺, z poziomami VEGF i angiogeniny oraz z parametrami oceniającymi funkcję skurczową LK.

WNIOSKI Wczesna drożność IRA i mniej nasilona martwica mięśnia sercowego wydają się istotniejsze dla funkcji LK ocenianej u pacjentów 6 miesięcy po STEMI niż mobilizacja komórek CD34⁺ oraz poziomy czynników angiogennych.

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