Matrix metalloproteinases and the activity of their tissue inhibitors in patients with ST-elevation myocardial infarction treated with primary angioplasty

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A b s t r a c t

Background: Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play a role in heart remodelling after acute myocardial infarction (MI). Their activity is connected with outcome and heart failure development. There is little data on MMP and TIMP activity changes in the setting of ST elevation MI (STEMI) treated with primary percutaneous coronary intervention (pPCI).

Aim: To assess the dynamics of activity of MMP-2 and MMP-9 and their endogenous inhibitors TIMP-1 and TIMP-2 in the course of invasive treatment of STEMI.

Methods: The study included 95 patients (age 61.8 ± 12.4 years; 35 women) treated with pPCI with stent implantation due to 100% closure of the target vessel in a setting of STEMI. We measured the activity of MMP-2 and MMP-9 (by zymography, expressed with arbitrary units, AU), CK-MB (U/L), troponin I (ng/mL), TIMP-1, TIMP-2 (ng/mL) concentrations in a peripheral blood before the pPCI, immediately after and 3, 6, 12, 24 and 48 h after the procedure. Left ventricular ejection fraction (LVEF) was estimated at the hospital discharge using the Simpson method. There were two control groups: 15 healthy persons and 15 patients with stable coronary artery disease matched for age and sex with the studied group.

Results: The abrupt opening of the target vessel did not produce an early increase in the activity of the MMPs. Their activity was high at the beginning and slowly lowered with time after pPCI so that at 12, 24 and 48 h after pPCI their activity was significantly lower than before and immediately after the pPCI ($p < 0.05$ for all comparisons). The abrupt opening of the target vessel did not produce significant changes in the TIMP concentration. Only the TIMP-1 showed a slow increase in concentration and achieved a significantly higher level 48 h after the procedure compared to its concentration before and immediately after pPCI ($p < 0.05$). In 14 patients (15% of the studied group), the post procedure TIMI flow was estimated as lower than 3 (TIMI 1 or 2). There was significantly higher MMP-9 activity in this group before, immediately after and up to 3 h after PCI compared to the group with good angiographic effect (TIMI = 3 after procedure). Patients with lowered LVEF ($< 50\%$) at hospital discharge had higher MMP-9 activity immediately after and 3 h after pPCI compared to patients with preserved LVEF. The same relation was observed for TIMP-2 level, where patients with a higher level before and immediately after pPCI had lowered LVEF at discharge.

Conclusions: 1. The activity level of MMP-2 and MMP-9 is elevated during the STEMI acute phase and falls 12 h after successful pPCI, while TIMP-1 concentration only rises 48 h after the procedure. 2. The abrupt opening of the target vessel in STEMI does not produce acute changes in MMP-2, MMP-9 activity or TIMP-1 and TIMP-2 concentration. 3. The 'no-reflow' phenomenon in STEMI patients occurs more often in those with higher MMP-9 activity before pPCI. 4. Lowered LVEF at hospital discharge is observed in patients with higher periprocedural MMP-9 activity and TIMP-2 level.

Key words: matrix metalloproteinases (MMP), tissue inhibitors of MMP, acute myocardial infarction, primary PCI

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INTRODUCTION

Matrix metalloproteinases (MMP) are a family of zinc-dependent endopeptidases including: MMP-1, MMP-2, MMP-3, MMP-9, MT1-MMP, ADAM17 and ADAMTS-13 [1]. All MMPs share the following functional features: they degrade extracellular matrix component; almost all of them are secreted in a latent proform and need to be activated for their proteolytic activity; they contain zinc in their active site; and they are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs) [2, 3]. The MMP family shares a similar basic domain structure and can be divided into four groups based on structure and *in vitro* substrate specific for different extracellular matrix components. The first group is known as collagenases; they include MMP-1, MMP-8, MMP-13 and all of them can cleave fibrillar collagens type I, II and III. The second group contains gelatinases including MMP-2 and MMP-9, which are known for their ability to degrade gelatines. The third group constitutes the stromelysins (MMP-3, -10 and -11). They are active against a broad spectrum of extracellular matrix components including proteoglycans, laminins, fibronectin, vitronectin and some types of collagens. The fourth group contains membrane-type MMPs (MT-MMP) which degrade several extracellular matrix components and are also able to activate other MMPs [2].

MMP activity is inhibited in the tissue specifically by TIMPs. They are a family of enzymes consisting of four structurally related members: TIMP-1, -2, -3 and -4. TIMP-1 potently inhibits the activity of all MMPs with the exception of MMP-2 and MT-MMP. TIMP-2 is a potent inhibitor of most MMPs except MMP-9. TIMP-3 inhibits MMP-1, -2, -3, -9 and -13, while TIMP-4 binds MMP-1, -3, -7 and -9 [2].

The level of activity of some MMPs can have an influence on inflammatory processes activity and atherosclerosis progression [4], as well as on the myocardial function after infarction (MI) [5], especially on the propensity for left ventricle (LV) free wall perforation in the course of ischaemia [6]. Furthermore, the imbalance between MMPs and TIMPs is the major factor responsible for cardiomyocyte and interstitial changes following acute MI in the infarcted and remote areas [7]. Recently, it has also been shown that plasma MMP-9 level can be associated with the extent of LV remodelling in a follow-up after acute MI.

Apart from this, to date there has been little data on the dynamics of MMP and TIMP activity in patients with acute ST-elevation MI (STEMI) treated with primary percutaneous coronary intervention (pPCI). Several studies have been published, but they differ as to the methodology of the MMPs and TIMPs measurement, inconsistent study design, and endpoints estimation.

The aim of our study was to assess the dynamics of activity of MMP-2 and MMP-9 and their endogenous inhibitors TIMP-1 and TIMP-2 in the course of invasive treatment of STEMI.

METHODS

Study group

The study included 95 consecutive patients with STEMI treated with pPCI. The control group consisted of 15 healthy volunteers and 15 patients with stable coronary artery disease (CAD). The pPCI was done using the femoral access and after standard projection angiography was done, target vessel was mechanically opened and following pre-dilatation stent was implanted to ensure vessel patency. The TIMI blood flow scale in the dilated vessel was used to assess the procedure success. TIMI < 3 in the target vessel after pPCI was classified as 'no-reflow', and TIMI = 3 was classified as a good PCI result. Additionally, in all patients left ventricular ejection fraction (LVEF) was estimated at hospital discharge using the Simpson technique.

Our study complied with the Declaration of Helsinki. The locally appointed ethics committee approved the research protocol, and informed consent was obtained from the subjects.

Study plan

Blood for the study was collected into probes with anticoagulant (sodium citrate 0.105 M solution) (Sarstedt, Germany) at the following time points: before pPCI, immediately after it, and three, six, 12, 24 and 48 hours later. The blood was centrifuged and then stored at −70°C for the further estimation of MMP-2, MMP-9, TIMP-1 and TIMP-2 level. At every time point blood was also collected for MB isoenzyme of creatinine kinase (CK-MB), troponin I and white blood cells (WBC) count.

The level of activity of pro-MMP-2 and pro-MMP-9 in the plasma was established using gelatin zymography [8]. For the purpose of method standardisation, in every gel a relative standard was included: this was the supernatant from the culture of the lung cancer A 549, which contains pro-MMP-9. The activity of enzymes was completely inhibited with 1 mM phenantroline. The scanned gels were analysed using Sigma Gel software (Jandel Scientific, USA). The activity of each enzyme was presented in arbitrary units (AU). Intra-assay and inter-assay coefficient of variation was below 15% and 25%, respectively.

The TIMP-1 and TIMP-2 concentration was established using the ELISA method with the use of commercially available kits (R&D, UK).

Statistical analysis

Data is presented as mean ± SD for the normally distributed parameters, or as a median and interquartile range for data showing departures from normality. Some data that showed a right-skewed distribution but met the remaining criteria for normal distribution was transformed logarithmically and analysed by relevant parametric tests. To compare raw and transformed data with distributions that according to Shapiro-Wilkinson's test were normal, we used Student's *t* test and

Table 1. Characteristics of the studied group, stable coronary artery disease (CAD) controls and healthy controls

Parameter	Studied group (n = 95)	Stable CAD (n = 15)	Healthy controls (n = 15)
Age [years]	61.8 ± 12.4	60.1 ± 8.1	57.3 ± 7.1
Women/men	35/60	5/10	5/10
Arterial hypertension	73 (77%)	13 (86%)	0 (0%)
Diabetes mellitus	21 (22%)	5 (30%)	0 (0%)
Active smokers	34 (36%)	4 (26%)	4 (26%)
History of PCI/CABG	32/10	13/2	0/0
Statin	95 (100%)	15 (100%)	–
Beta-blockers	83 (87%)	13 (86%)	–
ACE-I	85 (90%)	10 (66%)	–
ASA + clopidogrel	95 (100%)	ASA (100%)	–
Single vessel disease/multiple vessel disease	71/24	8/7	–
Target vessel: LAD/Cx/RCA	35/15/45	–	–
Pain duration up to pPCI [h]	5.07 ± 2.9	–	–
TIMI 0 before the pPCI	95 (100%)	–	–
TIMI = 3 after pPCI	81 (85%)	–	–
TIMI < 3 after pPCI	14 (15%)	–	–
LVEF at hospital discharge [%]	56.2 ± 11.6	–	–

ACE-I — angiotensin converting enzyme inhibitor; ASA — acetylsalicylic acid; LAD — left anterior descending artery; Cx — left circumflex coronary artery; RCA — right coronary artery; LVEF — left ventricular ejection fraction; PCI — percutaneous coronary intervention; TIMI — thrombolysis in myocardial infarction

one-way or two-way ANOVA. For the remaining variables, including those showing bimodal distributions, a Mann-Whitney U test was employed to assess the significance of differences between groups of non-paired data. Spearman's rank correlation (RS) (for testing the null hypothesis of independence between two variables) or Kendall's rank correlation (for testing the strength of dependence between variables) were used to assess simple associations.

RESULTS

The full characteristics of the studied group and controls are set out in Table 1. The changes in MMP-2 and MMP-9 activity are presented in Table 2 and Figures 1 and 2.

The level of activity of MMP-2 in the studied group was significantly lower compared to the control group of healthy volunteers 12, 24 and 48 hours after PCI ($p < 0.05$ for all comparisons) but not before or at three and six hours after pPCI. The level of activity of MMP-2 in the studied group was not significantly different from the values obtained in the control group of stable CAD patients in every time point.

The level of activity of MMP-9 in the studied group was significantly higher compared to values obtained in the control group of volunteers (31.0 ± 9.4 AU) and in the control group of stable CAD patients (62.5 ± 63.0 AU) in every time point ($p < 0.05$ for all comparisons). There were no significant differences between both control groups regarding MMP-2 and MMP-9 activity.

Table 2. Matrix metalloproteinase 9 and 2 (MMP-9 and MMP-2) activity in the studied group (n = 95)

Time point	MMP-2 [AU]	MMP-9 [AU]
Pre PCI	227.8 ± 75.7	340.2 ± 72.1
Post PCI	210.9 ± 79.0	325.4 ± 91.3
3 h post PCI	204.5 ± 88.0	250.7 ± 32.2
6 h post PCI	191.6 ± 63.0	257.1 ± 71.6
12 h post PCI	167.9 ± 81.0	202.5 ± 64.9
24 h post PCI	185.1 ± 68.0	182.3 ± 38.1
48 h post PCI	176.1 ± 76.0	150.3 ± 93.0
Controls		
Normal controls	217.3 ± 39.3	132.4 ± 82.6
Stable CAD controls	204.9 ± 59.7	190.7 ± 86.7

PCI — percutaneous coronary intervention; CAD — coronary artery disease; AU — arbitrary units

The abrupt opening of the target vessel did not produce an early increase of the MMPs activity. Their activity was high at the beginning and slowly lowered with time after pPCI, so that at 12, 24 and 48 hours after pPCI their activity was significantly lower than before and immediately after the pPCI ($p < 0.05$ for all comparisons). This tendency is shown in Figures 1 and 2.

The changes of TIMP-1 and TIMP-2 concentration are presented in Table 3 and Figures 3 and 4.

TIMP-1 level in the studied group was significantly lower at three hours after pPCI compared to the control group of
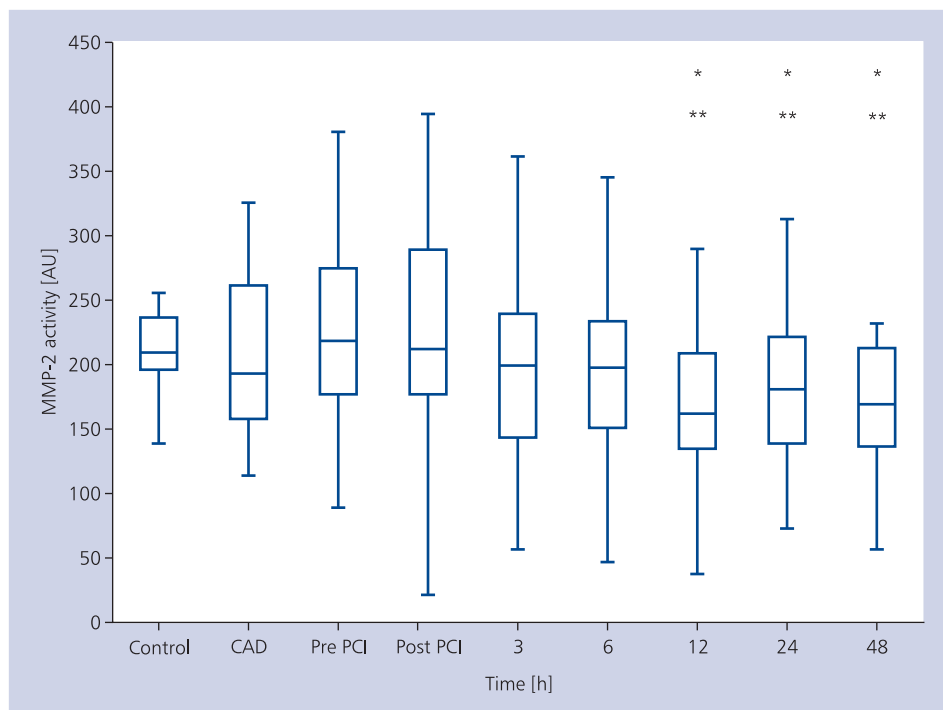
Figure 1. Changes in matrix metalloproteinase-2 (MMP-2) activity before and after primary percutaneous coronary intervention (PCI) and in controls. Data is median with upper and lower quartiles and range. Asterisk indicates p < 0.05 in comparison between control group of healthy volunteers and 12, 24 and 48 hour time points. Double asterisk indicates p < 0.05 in comparison between MMP-2 activity pre PCI and post PCI vs. 12, 24 and 48 hour time points; CAD — control group of patients with stable coronary artery disease; Control — control group of healthy volunteers
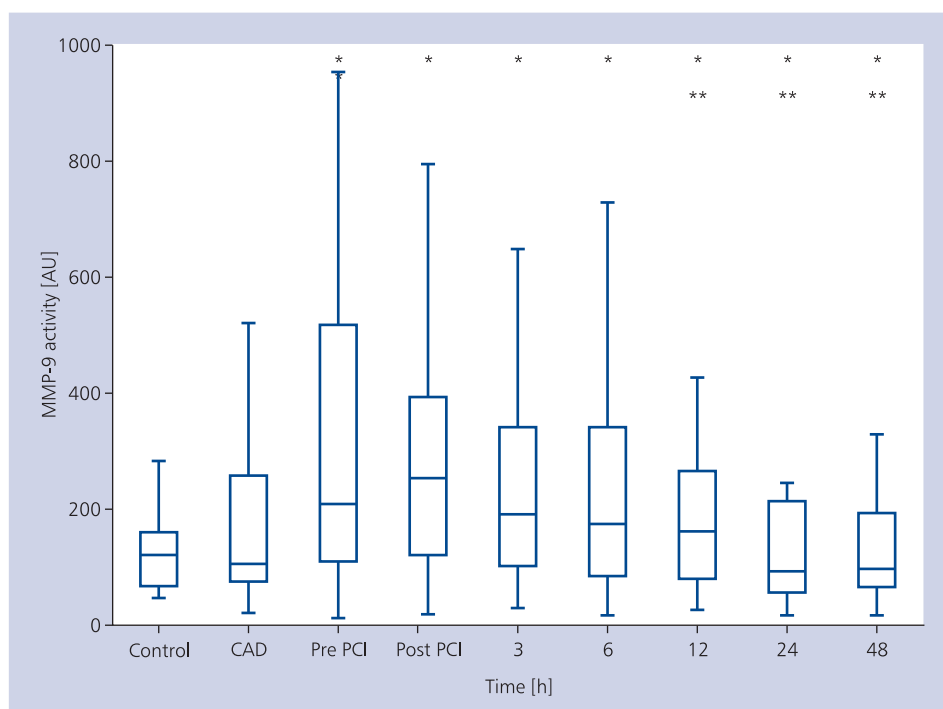

Figure 2. Changes in matrix metalloproteinase-9 (MMP-9) activity before and after primary percutaneous coronary intervention (PCI) and in controls. Data is median with upper and lower quartiles and range. Asterisk indicates p < 0.05 in comparison between control group of healthy volunteers and control group of stable coronary artery disease patients vs. all time points in studied group. Double asterisk indicates p < 0.05 in comparison between MMP-9 activity pre PCI and post PCI vs. 12, 24 and 48 hour time points; CAD — control group of patients with stable coronary artery disease; Control — control group of healthy volunteers

healthy volunteers and with the patients with stable CAD ($p < 0.05$ for both comparisons).

TIMP-2 concentration in the studied group was significantly lower in all time points compared to the control group of healthy volunteers and with the patients with stable CAD ($p < 0.05$ for both comparisons). There were no significant differences between both control groups as for TIMP-1 and TIMP-2 concentrations.

The abrupt opening of the target vessel did not produce significant changes in the TIMP concentration. Only the TIMP-1 showed a slow increase in concentration and achieved a significantly higher level 48 hours after the procedure compared to its concentration before and immediately after pPCI ($p < 0.05$).

We found no correlation between MMPs activity and TIMPs level throughout all time points. There was also no correlation between WBC, neutrophils and MMPs. We found no difference in MMPs activity and TIMPs level between single vessel disease and multi vessel disease patients.

We found also no correlation between CK-MB or troponin level and MMPs activity or TIMPs level in the group as a whole. The dynamics of the abovementioned substances varied importantly. A graphical presentation of the changes in TIMP concentration and MMP activity against the background of CK-MB and troponin dynamics is presented in Table 4 and Figure 5.

In 14 patients (15% of the studied group), the post procedure TIMI flow was estimated as lower than 3 (TIMI 1 or 2). This group was compared to patients in whom the TIMI flow after pPCI was equal to 3 (81 patients, 85% of the studied group). Statistically significant differences as for MMP and TIMP values were obtained only with MMP-9. The results of this analysis are presented in Table 5 and Figure 6.

Table 3. Tissue inhibitors of matrix metalloproteinases (TIMP-1 and TIMP-2) level in the studied group (n = 95)

Time point	TIMP-1 [ng/mL]	TIMP-2 [ng/mL]
Pre PCI	36.3 ± 15.3	43.1 ± 15.3
Post PCI	35.1 ± 16.3	42.6 ± 14.9
3 h post PCI	31.8 ± 12.2	34.9 ± 11.4
6 h post PCI	37.0 ± 17.9	38.9 ± 13.3
12 h post PCI	39.2 ± 26.8	32.3 ± 13.0
24 h post PCI	50.1 ± 22.3	39.7 ± 15.8
48 h post PCI	56.0 ± 38.8	39.7 ± 18.8
Controls		
Normal controls	41.0 ± 7.5	49.4 ± 20.4
Stable CAD controls	40.9 ± 13.3	51.8 ± 12.6

PCI — percutaneous coronary intervention; CAD — coronary artery disease
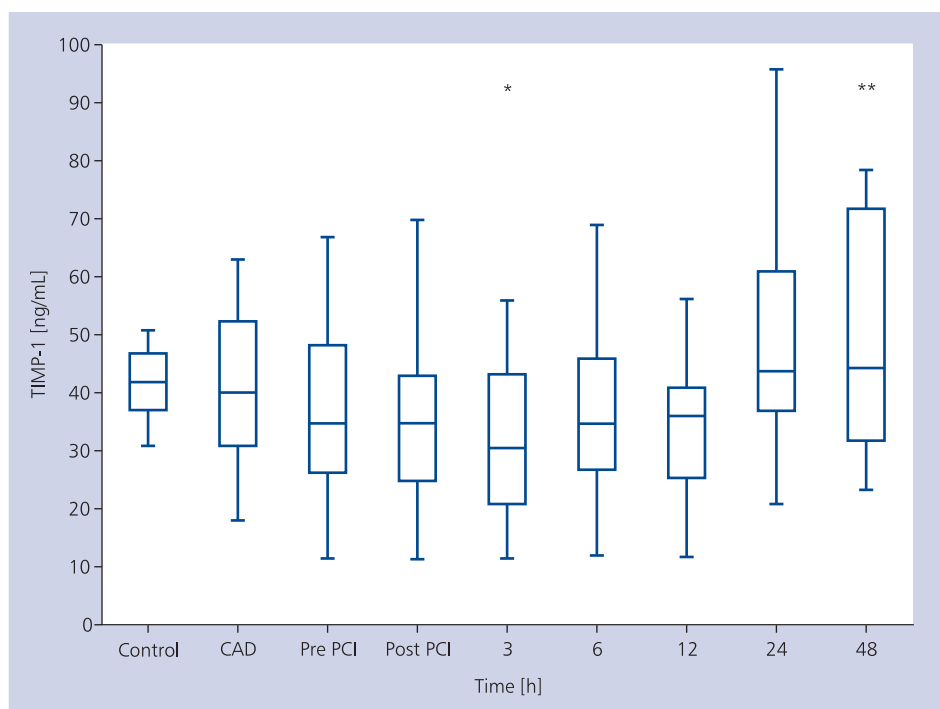

Figure 3. Changes in tissue inhibitors of matrix metalloproteinase-1 (TIMP-1) activity before and after primary percutaneous coronary intervention (PCI) and in controls. Data is median with upper and lower quartiles and range. Asterisk indicates $p < 0.05$ in comparison between control group of healthy volunteers and control group of stable coronary artery disease patients vs. TIMP-1 level three hours after primary PCI. Double asterisk indicates $p < 0.05$ in comparison between TIMP-1 level 48 hours after primary PCI vs. its level pre PCI and post PCI; CAD — control group of patients with stable coronary artery disease; Control — control group of healthy volunteers
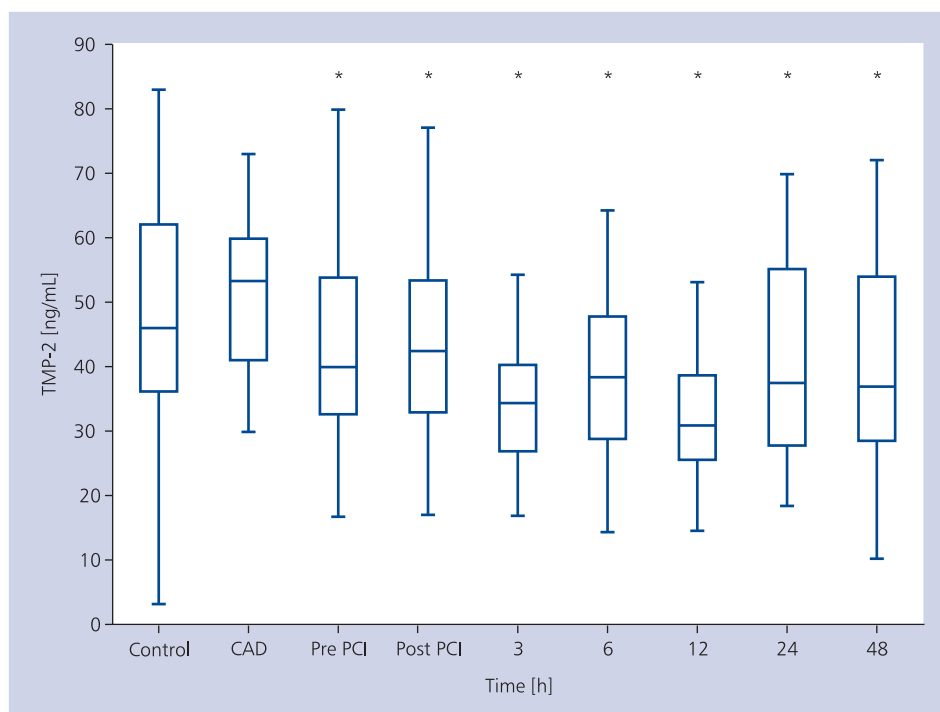
Figure 4. Changes in tissue inhibitors of matrix metalloproteinase-2 (TIMP-2) activity before and after primary percutaneous coronary intervention (PCI) and in controls. Data is median with upper and lower quartiles and range. Asterisk indicates $p < 0.05$ in comparison between control group of healthy volunteers and control group of stable coronary artery disease patients vs. all time points in studied group; CAD — control group of patients with stable coronary artery disease; Control — control group of healthy volunteers

Table 4. Troponin I (TnI) and CK-MB level in the studied group

Time point	TnI [ng/mL] N = 95	CK-MB [U/L] N = 95
Pre PCI	14.6 ± 41.2	101.5 ± 210.3
Post PCI	67.7 ± 92.0	214.8 ± 290.3
3 h post PCI	71.1 ± 67.6	264.3 ± 330.0
6 h post PCI	59.7 ± 51.7	218.4 ± 233.0
12 h post PCI	39.6 ± 38.3	161.2 ± 150.4
24 h post PCI	36.6 ± 36.0	92.2 ± 92.1
48 h post PCI	23.2 ± 20.0	48.4 ± 42.2

PCI — percutaneous coronary intervention; CK-MB — MB isoenzyme of creatinine kinase

Figure 5. Visual presentation of changes in tissue inhibitors of matrix metalloproteinases (TIMP) concentration and matrix metalloproteinases (MMP) activity on the background of MB isoenzyme of creatinine kinase (CK-MB) and troponin (TrI) dynamics. Points represent means. For better visualisation, standard deviations were omitted; PCI — percutaneous coronary intervention

Interestingly, there was significantly higher MMP-9 activity in the 'no-reflow' group (TIMI < 3 after procedure) before, immediately after, and up to three hours after PCI compared to the group with good angiographic effect (TIMI = 3 after procedure). Analysis of the receiver operator curve showed that MMP-9 activity before pPCI higher that 280 AU can predict no-reflow incidence with 87.5% sensitivity (95% CI 47.4–97.9) and 62.5% specificity (95% CI 48.5–75.1).

In the group of 'no-reflow' there was a significant ($p < 0.05$) correlation between MMP-9 activity immediately after pPCI and CK-MB l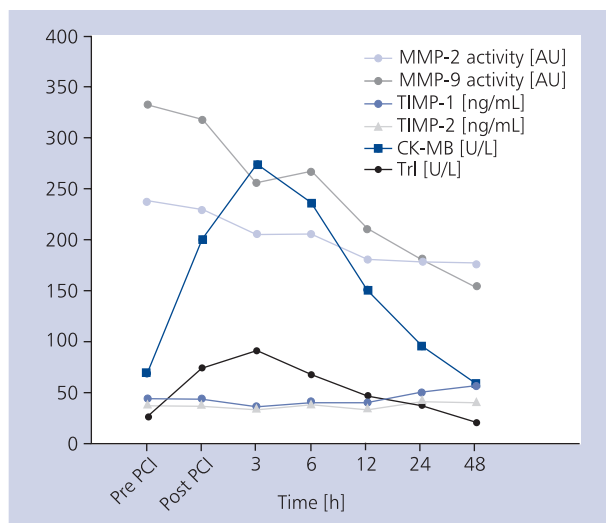evel at three time points: immediately after pPCI ($r = 0.88$), three hours after pPCI ($r = 0.82$) and six hours after pPCI ($r = 0.76$). Another significant correlation

Table 5. Matrix metalloproteinase 9 (MMP-9) activity in groups with and without 'no-reflow' phenomenon

Time point	MMP-9 in TIMI = 3 group [AU] N = 81	MMP-9 in TIMI < 3 group [AU] N = 14	Statistics
Pre PCI	275.1 ± 248.5	623.3 ± 424.1	p < 0.05
Post PCI	286.1 ± 244.9	504.1 ± 455.8	p < 0.05
3 h post PCI	236.2 ± 168.8	467.2 ± 494.1	p < 0.05
6 h post PCI	229.4 ± 263.8	354.8 ± 183.1	NS
12 h post PCI	195.2 ± 179.1	323.4 ± 294.9	NS
24 h post PCI	161.1 ± 256.7	188.5 ± 258.3	NS
48 h post PCI	107.2 ± 74.5	274.2 ± 226.0	NS

PCI — percutaneous coronary intervention; AU — arbitrary units
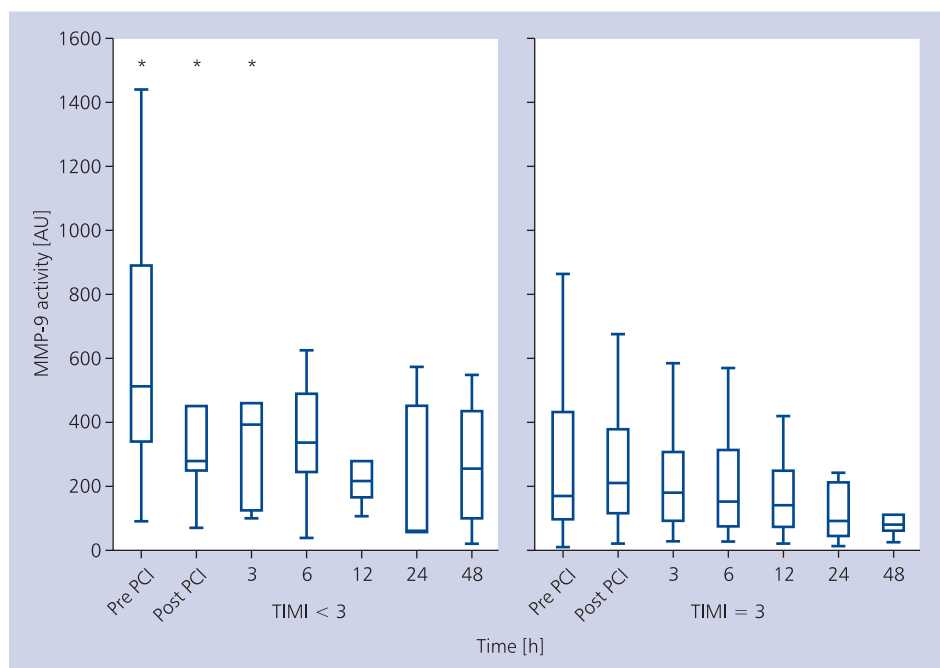

Figure 6. Co-existence of matrix metalloproteinase-9 (MMP-9) activity and 'no-reflow' phenomenon (TIMI < 3 after successful primary percutaneous coronary intervention [PCI]). Data is median with upper and lower quartiles and range. Asterisk indicates p < 0.05 in comparison between MMP-9 activity pre PCI, post PCI and three hours post PCI in TIMI < 3 group vs. MMP-9 activity pre PCI, post PCI and three hours post PCI in TIMI = 3 group

was found in the same group between TIMP-1 level 48 hours after pPCI and troponin level at the very beginning of hospitalisation; immediately after pPCI (r = 0.96), three hours after pPCI (r = 0.82), six hours after pPCI (r = 0.84), and 12 hours after pPCI (r = 0.79). There was also significant correlation in the 'no-reflow' group as for TIMP-2 level before pPCI and CK-MB level immediately after pPCI (r = 0.87), three hours after pPCI (r = 0.90) and six hours after pPCI (r = 0.78).

Further analysis showed another interesting relation between MMP-9 level and LVEF estimated at discharge. Patients with lowered LVEF (< 50%) at hospital discharge (median of seven days) had higher MMP-9 activity immediately after and three hours after pPCI compared to patients with preserved LVEF (MMP-9 activity [AU] 149.3 ± 162.7 vs. 71.9 ± 79.3 immediately after pPCI and 334.5 ± 542.6 vs. 108.8 ± 185.1 three hours after pPCI, p < 0.05 for both comparisons). The same relation was observed for TIMP-2 level, where patients with its higher level before and immediately after pPCI had lowered LVEF at discharge (TIMP-2 level [ng/mL] before pPCI 50.3 ± 13.9 vs. 39.4 ± 15.2 and 49.8 ± 15.4 vs. 39.0 ± 13.9 immediately after pPCI).

DISCUSSION

In our study we showed that the abrupt opening of the totally occluded coronary vessel due to acute MI does not provoke acute, comparable with necrotic enzymes, changes

in TIMP-1 and TIMP-2 concentration and MMP-2 and MMP-9 activity. The most pronounced effect was observed in the MMP-9 activity which was elevated in all time points compared to controls and slowly fell, reaching a significantly lower level of activity 12 hours after pPCI compared to the pre and post pPCI time points. As for MMP-2 activity, we found no significant elevation of it during the whole observation period, and values comparable with controls. There was a rather small but still significant fall in MMP-2 activity starting from 12 hours after pPCI compared to the pre and post pPCI state.

These findings are to some extent consistent with other studies. Wagner et al. [9] showed in 109 patients with STEMI treated with pPCI a significant elevation of MMP-9 activity (measured with gelatin zymography) during the first 24 hours from the syndrome start with a gradual fall of the activity during days 1, 2 and 3. In this study however there was performed only one measurement at admission which was inside the 24 hour window of the pain onset, and follow up measurements of MMP-9 activity were obtained in only 18% of the whole group (20 patients). Investigators found, as in our study, two groups of patients with relatively low (the bigger group) and relatively high MMP-9 activity at admission, but unlike in our study found no correlation between MMP-9 activity and final TIMI flow.

Our study, thanks to the full follow-up of all patients included and the strictly defined time points of blood sampling, was able to show that in patients with higher MMP-9 activity there is more risk for TIMI < 3 flow after the pPCI and that the elevated activity lasts up to three hours after the procedure. To the best of our knowledge, this is the first observation in the literature. This finding can be connected and explained mechanistically with that of Fukuda et al. [10] showing that in patients with elevated MMP-9 (in serum from peripheral blood) there was more often ruptured plaques at the intravascular ultrasound. MMP-9 origin from ruptured coronary plaques has also been confirmed by other groups [11–13]. Against the background of the above reports, the finding in our study that higher MMP-9 at admission could be connected with a higher likelihood of no-reflow syndrome could be due to more 'aggressive' plaques with more MMP-9 content and a bigger burden of possible material and substances to produce no-reflow after pPCI. Kelly et al. [14] studied the MMP-9 and MMP-2 level (estimated with ELISA) dynamics in 77 patients with STEMI, some (59%) treated with fibrinolysis and others with medical therapy. There was significant elevation of MMP-9 level inside the 0–12 hours from admission, with a significant fall at 12–24 hours, which is consistent with our finding, although we measured activity, not the concentration of MMP-9 and we had patients treated with pPCI. MMP-2 level in this study was higher that control in all time points (until 96 hours post admission) with no significant fall or increase inside the group. The difference with our study could be due to the analytical factor (we measured activity not the concentration), and/or the different treatment of patients (we performed pPCI in all patients, not thrombolysis in some of them). The better and more modern treatment in our group, with visual checking of the results obtained as for vessel patency (in the abovementioned study there is no information on the success of the thrombolysis), could lead to the comparable dynamics of the MMP-2 compared to MMP-9 where there was a gradual fall in its activity after successful pPCI.

Eckart et al. [15] studied the serum (not plasma) level of MMP-2 and MMP-9 in 100 patients with suspected acute coronary syndrome. Blood was drawn before, immediately after, and 24 hours after cardiac catheterisation and pPCI where appropriate. There was not significantly different MMP-2 level compared to control in estimated time points and there was a tendency to lower MMP-9 over time, with significant elevation in the subgroup of patients treated with pPCI. Also in this study there was comparable with our finding of MMP-2 being rather outside the control range and MMP-9 falling over time. There was though a significant increase in MMP-9 level not observed in our study after pPCI, but analytical differences and no data on the pPCI success render comparison with our study difficult.

It should be pointed out that to best of our knowledge compared to available data our study is the biggest so far to estimate MMP and TIMP dynamics in a homogenous group of patients treated with the same procedure for the same disease — STEMI. Also in our study we showed for the first time that there is no acute fall or drop in MMP-2 and MMP-9 activity after abrupt opening of the occluded coronary vessel. Furthermore, there is significantly higher MMP-9 activity, especially before pPCI, in patients who further will develop no-reflow syndrome.

We showed that TIMP-1 level is significantly lower around three hours after primary pPCI and after 48 hours starts to be higher than in the controls. It seems that it is being used during the very acute phase and then starts to be delivered to counteract MMPs, yet this action in somehow moved in time compared to the MMPs activity dynamics. TIMP-1 potently inhibits the activity of most MMPs, with the exception of MMP-2 [2]. Some investigators did not find TIMP-1 in coronary plaques [11], yet others found it [12]. Tziakas et al. [16] found an elevated level of TIMP-1 in peripheral blood as we did, but already at admission with the peak level after 24 hours. In this study there was a heterogeneous population of 46 patients with MI treated with thrombolysis or glycoprotein IIb/IIIa without pPCI and the TIMP-1 level was measured in serum, not plasma as in our study, so the results are only barely comparable.

It is possible that measuring TIMP concentration further would show significant changes in their concentrations. This was the case in the study of Inocubo et al. [12] where there was, as in our study, a significant increase in TIMP-1 level in plasma two days after pPCI in acute coronary syndrome

patients, with sustained high levels up to day 21 after pPCI. A possible similar profile was observed by Webb et al. [17] where TIMP-1 level was above the normal range as soon as 24 hours after MI treated with pPCI.

As for TIMP-2 level, it was consistently lower throughout all time points compared to the controls. TIMP-2 is a potent inhibitor of most MMPs, except MMP-9 [2]. In our concept of the study, this inhibitor could be connected with MMP-2, but there was no correlation between them. We observed small, yet significant, changes of MMP-2, but TIMP-2 did not follow them as to some extent was observed in the MMP-9/TIMP-1 couple. There have been very few studies looking at TIMP-2 level before and some hours after pPCI in acute MI. Webb et al. [17] showed that TIMP-2 is elevated significantly compared to controls only 180 days after acute MI treated with pPCI. Until that day, starting from day one after pPCI or thrombolysis, MMP-2 level remained inside the reference range. This is somewhat different from the results of our study, where MMP-2 is below the levels obtained in controls. This can be in part explained by the number of patients included (n = 32) and inhomogeneous treatment provided for patients in the abovementioned study, where two thirds were treated with pPCI and the rest with thrombolysis, without data on treatment success. In this regard, our result could be of value because it gives information on the MMP-2 level in one well defined group of patients.

The results of our study nevertheless show that TIMP-1 and TIMP-2 concentrations rise more slowly than MMP-2 and MMP-9 fall, which could have an influence on the myocardial rearrangement after acute phase of STEMI.

The dynamics of cardiac necrotic enzymes in STEMI (troponin and CK-MB) are different from MMPs and TIMPs. In the studied group as a whole, we found no significant correlations between those two groups of markers. Nevertheless, in the 'no-reflow' group, although small (14 patients), we detected significant correlations. There were significant correlations between MMP-9 activity immediately after pPCI and CK-MB level immediately after pPCI and three and six hours later. There was also a correlation between TIMP-2 level before pPCI and CK-MB at the same time points as above. Finally, there was correlation between TIMP-1 level 48 hours after pPCI and troponin level immediately after, and three, six and 12 hours after pPCI. These correlations could be due to the fact that in patients with 'no-reflow' there is so much destruction of myocardium that MMPs and TIMPs go the same way as necrotic enzymes but with a time shift due to the overall differences in their dynamics. In this regard, especially TIMP-1 level 48 hours after pPCI would be presumably a 'healing response' to the high troponin level up to 12 hours after pPCI. Nevertheless, it should be kept in mind that these correlations are derived from a small subgroup analysis and their force is also small and could be due to chance.

In our study, we showed that patients with LVEF < 50% at discharge had higher activity of MMP-9 immediately after and three hours after pPCI compared to patients with preserved LVEF at discharge. These results are somewhat consistent with two studies in patients with acute STEMI where the peak serum MMP-9 level correlated with echocardiographic and neurohormonal measurements of LV dysfunction six weeks [18] and a median of 25 weeks after acute MI [14]. In another study, there was observed a positive correlation between MMP-2 and MMP-9 activity after two weeks in acute MI patients with successful coronary angioplasty as well as changes in LV volume in the next six months [19]. In a recent study by Szulik et al. [20] it was further shown that resynchronisation therapy in a group of patients with ischaemic heart failure ameliorates cardiac function which goes together with MMP-9 level lowering.

TIMP-2 level was not explored in terms of its influence on LVEF in the setting of acute MI. We showed that patients with LVEF < 50% at discharge had a higher TIMP-2 level before and immediately after pPCI compared to patients with preserved LVEF at discharge. It has to be noted that TIMP-2 cannot counteract the action of MMP-9, so according to our results it could be considered as a marker of elevation of other MMPs which it blocks [2]. Their higher activity (excluding MMP-2, which has no relation to LVEF in our study) could lead to more vigorous myocardial rearrangements, resulting in higher MMP-2 expression and worse LV outcome several days after successful pPCI in acute MI.

CONCLUSIONS

1. The activity level of MMP-2 and MMP-9 is elevated during STEMI acute phase and falls 12 hours after successful pPCI, while TIMP-1 concentration rises only 48 hours after the procedure.
2. The abrupt opening of the target vessel in STEMI does not produce acute changes in MMP-2, MMP-9 activity and TIMP-1 and TIMP-2 concentration.
3. The 'no-reflow' phenomenon in STEMI patients occurs more often in those with higher MMP-9 activity before pPCI.
4. Lowered LVEF at hospital discharge is observed in patients with higher periprocedural MMP-9 activity and TIMP-2 level.

Conflict of interest: *none declared*

References

1. Fingleton B. Matrix metalloproteinases as valid clinical targets. Curr Pharm Des, 2007; 13: 333–346.
2. Phatharajaree W, Phrommintikul A, Chattipakorn N. Matrix metalloproteinases and myocardial infarction. Can J Cardiol, 2007; 23: 727–733.
3. Santos-Martinez MJ, Medina C, Jurasz P, Radomski MW. Role of metalloproteinases in platelet function. Thromb Res, 2008; 121: 535–542.
4. Beaudeux JL, Giral P, Bruckert E et al. Matrix metalloproteinases, inflammation and atherosclerosis: therapeutic perspectives. Clin Chem Lab Med, 2004; 42: 121–131.
5. Ducharme A, Frantz S, Aikawa M et al. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlarge-

ment and collagen accumulation after experimental myocardial infarction. J Clin Invest, 2000; 106: 55–62.

6. Hayashidani S, Tsutsui H, Ikeuchi M et al. Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction. Am J Physiol Heart Circ Physiol, 2003; 285: H1229–H1235.

7. Mukherjee R, Brinsa TA, Dowdy KB et al. Myocardial infarct expansion and matrix metalloproteinase inhibition. Circulation, 2003; 107: 618–625.

8. Kleiner DE, Stetler-Stevenson WG. Quantitative zymography: detection of picogram quantities of gelatinases. Anal Biochem, 1994; 218: 325–329.

9. Wagner DR, Delagardelle C, Ernens I et al. Matrix metalloproteinase-9 is a marker of heart failure after acute myocardial infarction. J Card Fail, 2006; 12: 66–72.

10. Fukuda D, Shimada K, Tanaka A et al. Comparison of levels of serum matrix metalloproteinase-9 in patients with acute myocardial infarction versus unstable angina pectoris versus stable angina pectoris. Am J Cardiol, 2006; 97: 175–180.

11. Fiotti N, Altamura N, Orlando C et al. Metalloproteinases-2, -9 and TIMP-1 expression in stable and unstable coronary plaques undergoing PCI. Int J Cardiol, 2008; 127: 350–357.

12. Inokubo Y, Hanada H, Ishizaka H et al. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. Am Heart J, 2001; 141: 211–217.

13. Chen F, Eriksson P, Hansson GK et al. Expression of matrix metalloproteinase 9 and its regulators in the unstable coronary atherosclerotic plaque. Int J Mol Med, 2005; 15: 57–65.

14. Kelly D, Cockerill G, Ng LL et al. Plasma matrix metalloproteinase-9 and left ventricular remodelling after acute myocardial infarction in man: a prospective cohort study. Eur Heart J, 2007; 28: 711–718.

15. Eckart RE, Uyehara CF, Shry EA et al. Matrix metalloproteinases in patients with myocardial infarction and percutaneous revascularization. J Interv Cardiol, 2004; 17: 27–31.

16. Tziakas DN, Chalikias GK, Parissis JT et al. Serum profiles of matrix metalloproteinases and their tissue inhibitor in patients with acute coronary syndromes. The effects of short-term atorvastatin administration. Int J Cardiol, 2004; 94: 269–277.

17. Webb CS, Bonnema DD, Ahmed SH et al. Specific temporal profile of matrix metalloproteinase release occurs in patients after myocardial infarction: relation to left ventricular remodeling. Circulation, 2006; 114: 1020–1027.

18. Squire IB, Evans J, Ng LL et al. Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction. J Card Fail, 2004; 10: 328–333.

19. Matsunaga T, Abe N, Kameda K et al. Circulating level of gelatinase activity predicts ventricular remodeling in patients with acute myocardial infarction. Int J Cardiol, 2005; 105: 203–208.

20. Szulik M, Stabryla-Deska J, Boidol J et al. Echocardiography-based qualification and response assessment to cardiac resynchronisation therapy in patients with chronic heart failure. The matrix metalloproteinase-9 substudy. Kardiol Pol, 2011; 69: 1043–1051.

Aktywność metaloproteinaz macierzy i ich inhibitorów u pacjentów z zawałem serca z uniesieniem odcinka ST leczonych pierwotną angioplastyką wieńcową

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Streszczenie

Wstęp: Metaloproteinazy macierzy (MMP) i ich inhibitory (TIMP) uczestniczą w przebudowie mięśnia sercowego po zawale serca (MI). Ich aktywność wiąże się z rokowaniem odległym i rozwojem niewydolności serca. Niewiele natomiast wiadomo o dynamice zmian stężeń tych substancji w ostrej fazie MI leczonego pierwotną interwencją wieńcową (pPCI).

Cel: Celem badania była ocena dynamiki zmian aktywności MMP-2, MMP-9 oraz ich inhibitorów 1 i 2 (TIMP-1, TIMP-2) w trakcie i w okresie okołozabiegowym leczenia inwazyjnego pacjentów z MI z uniesieniem odcinka ST (STEMI).

Metody: Do badania włączono kolejnych pacjentów ze STEMI leczonych pPCI z implantacją stentu. Metodą zymograficzną oceniano aktywność MMP-2 i MMP-9 (AU) oraz stężenia CK-MB (j./l), troponiny I (ng/ml), TIMP-1, TIMP-2 (ng/ml) we krwi obwodowej przed pPCI, bezpośrednio po zakończeniu zabiegu, następnie 3, 6, 12, 24 i 48 godzin po zabiegu. Przy wypisie ze szpitala oceniano ultrasonograficznie frakcję wyrzutową lewej komory (LVEF). Dwie grupy kontrolne składały się z 15 zdrowych ochotników i 15 pacjentów ze stabilną chorobą niedokrwienną serca dobranych z grupą badaną pod względem płci i wieku.

Wyniki: Do badania włączono 95 pacjentów (wiek 61,8 ± 12,4 roku; w tym 35 kobiet). Nagłe udrożnienie naczynia dozawałowego nie spowodowało wczesnego wzrostu aktywności MMP. Aktywność ta była wysoka w początkowym okresie MI i stopniowo spadała tak, że 12, 24 i 48 godzin po pPCI była istotnie niższa w porównaniu z okresem przed i po zakończeniu pPCI (p < 0,05). Nagłe udrożnienie naczynia dozawałowego nie spowodowało również istotnych zmian w stężeniu TIMP. Stężenie TIMP-1 wykazywało powolny trend wzrostowy i w 48. godzinie po pPCI było istotnie wyższe w porównaniu ze stężeniem przed i bezpośrednio po zakończeniu pPCI. U 14 pacjentów (15% grupy badanej), u których stwierdzono po pPCI wskaźnik TIMI < 3, wykryto istotnie wyższy poziom aktywności MMP-9 przed pPCI oraz bezpośrednio i 3 godziny po zabiegu w porównaniu z pacjentami z przepływem TIMI = 3 (p < 0,05). Pacjenci z LVEF < 50% przy wypisie ze szpitala charakteryzowali się wyższą aktywnością MMP-9 i stężeniem TIMP-2 bezpośrednio i 3 godziny po pPCI w porównaniu z pacjentami z LVEF > 50% (p < 0,05).

Wnioski: 1. Aktywność MMP-2 i MMP-9 jest podwyższona w trakcie STEMI i zmniejsza się 12 godzin po pPCI, podczas gdy stężenie TIMP-1 wzrasta po 48 godzinach od pPCI. 2. Udrożnienie naczynia dozawałowego w STEMI nie prowadzi do nagłych zmian w aktywności MMP-2, MMP-9 oraz stężeń TIMP-1 i TIMP-2. 3. TIMI < 3 stwierdza się częściej u pacjentów z wyższą okołozabiegową aktywnością MMP-9. 4. Obniżona LVEF przy wypisie ze szpitala występuje częściej u pacjentów z okołozabiegowym podwyższeniem aktywności MMP-9 i stężenia TIMP-2.

Słowa kluczowe: metaloproteinazy macierzy, tkankowe inhibitory metaloproteinaz tkankowych, zawał serca, pierwotna angioplastyka wieńcowa

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