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Endothelin-1 and nitric oxide in 3-year prognosis after acute myocardial infarction

Endotelin-1 i azot-monoksid u trogodišnjoj prognozi nakon akutnog infarkta miokarda

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Abstract

Background/Aim. Acute myocardial infarction (AMI) is an important cause of mortality/morbidity worldwide. Biomarkers improve diagnostic and prognostic accuracy in AMI. The aim of this study was to investigate an increase of markers of endothelial dysfunction in AMI, measured on the 3rd day after the initial event and to investigate their association with short- and long-term (3-year) prognosis (outcome). **Methods**. The prospective study included 108 patients with AMI in the experimental group and 50 apparently healthy subjects in the control group. Endothelin-1 (ET-1) and nitric oxide degradation products (NOx) were determined. **Results.** The average age of the participants in the experimental group was 62 ± 10 years and 59 ± 9 years in the control group; 74.1% of the patients in experimental group were males and 68.8% in the control group. In 74.1% of the patients, ST-elevation myocardial infarction (STEMI) was diagnosed, and 25.9% of the patients presented with non-ST-elevation myocardial infarction (NSTEMI). Thirteen (5.6%) patients died during 3 years and they had significantly higher ET-1 levels compared to survivors [4.02 (2.72–5.93) *vs* 3.06 (2.23–3.58) pg/mL; $p = 0.015$]. Endothelin-1 in 46 (42.6%) patients with composite endpoint (3-year mortality and rehospitalization) was significantly increased compared to other patients [3.14 (2.54–4.41) *vs* 3.05 (2.18–3.56) pg/mL; $p = 0.035$]. Intrahospital complications were found in 41 (48%) patients. Participants with echocardiographically detected complications (ventricular dyskinesia, left ventricular thrombus and papillary muscle rupture) had higher ET-1 levels compared to other patients [4.02 (2.78–5.57) *vs* 3.06 (2.29–3.66) pg/mL; $p = 0.012$]. Endothelin-1 concentration above the 75th percentile (> 3.77 pg/mL) was associated with the increased risk for composite endpoint [Log Rank ($\chi^2 = 13.44$; $p < 0.001$)]. Patients who were rehospitalized had significantly lower NOx concentration [125.5 (111.4–143.6) *vs* 139.3 (116.79–165.2) μmol/L; $p = 0.04$]. Endothelin-1 positively correlated with high sensitivity troponin I (hsTnI), brain natriuretic peptide (BNP) and a number of leukocytes. **Conclusion.** Endothelin-1 and NOx were increased on the 3rd day after AMI, and they were predictors of worse short- and long-term (3-year) prognosis (outcome). Endothelin-1 positively correlated with conventional prognostic markers in AMI.

Key words:
myocardial infarction; biological markers; ultrasonography; endothelins; nitric oxide; prognosis; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Akutni infarkt miokarda (AIM) je značajan uzrok obolevanja i umiranja u svetu. Biomarkeri imaju značaj u postavljanju dijagnoze i u prognozi kod bolesnika sa AIM. Cilj studije bio je utvrđivanje porasta markera endotelne disfunkcije kod bolesnika sa AIM, merenih trećeg dana, poređuju sa vrednostima u kontrolnoj grupi zdravih ispitanika i značaja pomenutih markera u kratkoročnoj i dugoročnoj (trogodišnjoj) prognozi (ishodu). **Metode.** U prospektivnu studiju bilo je uključeno 108 bolesnika sa AIM koji su činili eksperimentalnu grupu i 50 zdravih dobrovoljaca kontrolne grupe. Kod svih ispitanika određivani su endotelin-1 (ET-1) i degradacioni produkti azot-monoksida (NOx). **Rezultati.** Prosečna starost ispitanika u eksperimentalnoj grupi bila je 62 ± 10 godina, a 59 ± 9 godina ispitanika u kontrolnoj grupi. U eksperimentalnoj grupi 74.1% ispitanika bilo je muškog pola a 68.8% u kontrolnoj grupi. Kod 74.1% bolesnika iz eksperimentalne grupe postavljena je dijagnoza infarkta miokarda sa elevacijom ST segmenta (STEMI), dok je 25.9% imalo infarkt miokarda bez delovanja ST segmenta (NSTEMI). Tokom 3 godine praćenja umrlo je 13 (5.6%) bolesnika. Oni su imali više koncentracije ET-1 u poređenju

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sa preživelim bolesnicima [4,02 (2,72–5,93) *vs* 3,06 (2,23–3,58) pg/mL; $p = 0,015$]. Zajedno, mortalitet i rehospitalizacija bili su prisutni kod 46 (42,6%) bolesnika, koji su takođe imali više koncentracije ET-1 [3,14 (2,54–4,41) *vs* 3,05 (2,18–3,56) pg/mL; $p = 0,035$]. Intrahospitalne komplikacije bile su prisutne kod 41 (48%) bolesnika, a oni sa ehokardiografski uočenim komplikacijama (ventrikularna diskinezija, tromb u levoj komori i ruptura papilarnog mišića) imali su značajno viši ET-1 [4,02 (2,78–5,57) *vs* 3,06 (2,29–3,66) pg/mL; $p = 0,012$]. Vrednosti ET- 1 iznad 75-og percentila (> 3,77 pg/mL) bile su udružene sa povećanim rizikom od lošeg ishoda [Log Rank (χ^2=13,44; $p < 0,001$)]. Bolesnici koji su rehospitalizovani imali su niže vrednosti NOx [125,5 (111,4–143,6) *vs* 139,3 (116,79–165,2) μmol/L; $p = 0,04$]. Vrednosti endotelina-1 bile su u pozitivnoj korelaciji sa visoko-senzitivnim troponinom I (hsTnI), moždanim natriuretskim peptidom (BNP) i brojem leukocita. **Zaključak.** Endotelin-1 i NOx su bili povišeni trećeg dana od AIM i bili su pokazatelji loše kratkotrajne (intrahospitalne) i trogodišnje prognoze ishoda. Vrednosti endotelina-1 bile su u korelaciji sa tradicionalnim prognostičkim markerima u AIM.

Ključne reči:
infarkt miokarda; biološki pokazatelji; ultrasonografija; endotelini; azot, oksidi; prognoza; osetljivost i specifičnost.

Introduction

Almost seven million people or 12.8% of the whole human population die due to coronary artery disease (CAD) during one year [1].

The endothelium is included in the development of atherosclerosis since it has a role in the maintenance of the vascular tonus, regulation of platelet and leukocyte activity and thrombosis/thrombolysis. Increased oxidative stress is closely related to endothelial dysfunction during the process of atherogenesis and development of complications such as acute myocardial infarction (AMI) [2–4].

Vascular smooth muscle tonus and leukocyte and platelet activity are controlled by the endothelium through a release of different mediators such as nitric oxide (NO) or endothelin-1 (ET-1) [5]. Nitric oxide and ET-1 are in physiological equilibrium in normal vasculature and low NO production or increases of ET-1 levels initiate endothelial dysfunction [6].

Endothelin-1 is a small peptide and it originates from the precursor called preproendothelin-1 (212 amino-acids). After removing its signaling portion and after cleavage action of endothelin converting enzyme, preproendothelin-1 forms two molecules: active ET-1 (with 21 amino acids) and inactive C-terminal part. Endothelin-1 is normally present at very small concentrations in the blood due to its quick reaction with receptors and due to the action of plasma neutral endopeptidases. Its half-life is estimated to be only 2 minutes [7, 8]. Endothelin-1 was first isolated from the porcine aortic endothelium. It is the most powerful vasoactive biomolecule isolated so far [9]. In AMI level of ET-1 in the blood rapidly increases. In patients without complications, the peak of ET-1 concentration is achieved approximately six hours after chest pain onset and afterwards, ET-1 starts to decrease to its normal values [10].

Nitric oxide (NO) is a free radical biosynthesized by the various forms of NO synthase (NOS) using L-arginine as a substrate. Nitric oxide has a short half-life *in vivo* and its degradation forms: nitrites and nitrates (NOx) that are used for its indirect measurement. Under normal physiological conditions, NO mediates many actions of the endothelium. Nitric oxide promotes relaxation in the vascular smooth muscle cells and has antithrombotic and antiatherogenic properties [11, 12]. In patients with stable CAD, NOx levels are decreased, however during the AMI inducible NOS (iNOS) from leukocytes is stimulated and therefore NOx concentrations are higher [11].

It seems that in AMI, endothelin-1 and NO exert the best predictive role when they are measured 48h after MI onset [11, 13].

The aim of our study was to determine is there an increase in markers of endothelial dysfunction (ET-1 and NOx) on the 3rd day after AMI. The second aim was to investigate the association of those biomarkers with short and long-term (3-year) prognosis (outcome).

Methods

In the prospective study 108 patients admitted to the Coronary Care Unit (CCU) at Clinic for Cardiovascular Diseases, Clinical Center Nis, during April–June 2012, without previously diagnosed diabetes mellitus, chronic kidney disease, and connective tissue disorders or other severe chronic diseases requiring treatment were included in the experimental (AMI) group. Control group comprised of 50 apparently healthy, age and gender matching, volunteer blood donors from the Blood Transfusion Institute, Niš. Subjects who were willing to participate in the study were scheduled for ambulatory physical and resting electrocardiographic (ECG) examination when detailed medical history was taken and blood samples were drawn. Volunteers with detected abnormalities were sent to primary care institutions for further diagnostic and therapeutic interventions. Diagnosis of AMI was made according to the European Society of Cardiology Guidelines [14].

The study was conducted in accordance with the Helsinki Declaration and Regional Ethics Committee approval. Prior to the study inclusion, all participants signed the informed consent.

For biomarkers measurement, venous blood samples from patients with AMI were collected on the 3rd day after admission to the CCU. In the control group, sampling was done in the morning, after 12 hours of fasting and no nicotine use. Plasma samples were stored at -20ºC. Endotelin-1 was measured by ELISA method with commercial Quantikine Endothelin-1 Immunoassay test from R&D Systems (USAR&D Company Minneapolis). Assay sensitivity is 0.207 pg/mL, and the assay range is 0.390–25 pg/mL. The

coefficient of variation for intra-assay precision in three different samples was 4%, 2.3%, and 1.9%. Concentrations of NOx**:** nitrites/nitrates (NO_2^- /NO_3^-) were measured using the modified cadmium-reduction method of Navaro-Gonzalez et al. [15] based on the Griss reaction. The within-day (control plasma sample, n = 7) and between-days coefficient of variation (the same sample for 5 consecutive days) were 7% and 8.6%, respectively. Recoveries of both nitrites and nitrates in our samples were greater than 95% and the detection limit of the assay was 2.5 μmol/L.

Blood samples for routine biochemical and hematology analysis, including brain natriuretic peptide (BNP) and the high sensitivity C-reactive protein (hsCRP) were taken at the admission according to the protocol of the Clinic. Blood samples for the high sensitivity troponin I (hsTnI) were taken at least 6 hours after the symptom onset. Levels of BNP, hsCRP, and hsTnI were measured according to the description of the manufacturer [BNP (ARCHITECT assay, Abbott, USA), hsCRP (BECKMAN COULTER, USA), hsTnI (ARCHITECT STAT High sensitive Troponin-I assay, Abbott Diagnostics, USA)] at the Central Biochemical Laboratory, Clinical Center Niš.

All patients underwent a complete echocardiographic examination during 48 h after admission.

Ambulatory follow-up visits were scheduled on every 6 months. Subjects from the control group were contacted by phone. For those who died during the follow-up period, data were obtained from the family physician and death certificate.

All statistical calculations were performed using appropriate (non)parametric tests after verification of parameter distribution in each group. All comparisons between subgroups were performed using the Mann Whitney test, or ANOVA when appropriate. Spearman's rank correlation coefficient and Pearson's bivariate correlation analysis were used to investigate the relationship between two comparable variables. Variables without normal distribution (ET-1, NOx, hsTnI, BNP, hsCRP) were transformed to their natural logarithms for logistic regression analysis. Odds ratios refer to 1 standard deviation (SD) in the natural logarithmic scale. All data were presented as means ± SD or medians with range. The $p < 0.05$ was considered as significant. All statistical calculations were done using "SPSS 17.0 for Windows" (SPSS Inc., USA).

Results

The average age of participants in the study was 62 ± 10 years in the experimental and 59 ± 9 in the control group, 74.1% being males in the experimental and 68.8% in the control group.

In 74.1% of patients, ST-elevation myocardial infarction (STEMI) was diagnosed, and 25.9% of patients presented with non-ST-elevation myocardial infarction (NSTEMI). In 68.7% of patients with STEMI, the primary percutaneous coronary intervention (PCI) was performed, 27.9% received thrombolytic therapy (rt-PA), and in 3.4% rescue PCI was performed. Left ventricular ejection fraction (LVEF) was 52.59 ± 10.54% in the experimental and 64.33 ± 18.63% in the control group. Clinical and laboratory characteristics of participants are shown in Tables 1 and 2.

Plasma levels of ET-1 ranged from 1.86 to 6.27 pg/mL in the experimental, and from 1.18 to 4.63 pg/mL in the control group, with a median and inter-quartile range (IQR) presented in Table 2. Nitric oxide degradation products were significantly higher in AMI compared to the control group ($p < 0.001$) (Table 2 and Figure 1).

Forty-one (48%) patients had intrahospital complications. Echocardiographically detected complications: ventricular dyskinesia, left ventricular thrombus and papillary muscle rupture were found in 9 (8.3%) patients and 5 (4.6%) had myocardial re-infarction. Arrhythmias and conductance disturbances were found in 24 (22%) patients. They included ventricular fibrillation, ventricular tachycardia, paroxysmal atrial fibrillation and other forms of supraventricular

Table 1

Clinical characteristics of participants

Parameters	Experimental group n (%)	Control group n (%)
Age (years), x̄ ± SD	62.44 ± 10.42	59.37 ± 9.46
Gender (males)	80 (74.1)	34 (68.8)
Arterial hypertension	82 (75.9)	/
Dyslipidemia		
no	51 (47.2)	50 (100)
yes and treated	45 (41.7)	/
yes and not treated	12 (11.1)	/
Smoking		
never	29 (26.9)	18 (66)
current	51 (47.2)	15 (30)
stopped	28 (25.9)	2 (4)
Diabetes mellitus *de novo*	10 (9.25)	/
Impaired fasting glucose	6 (5.55)	/
Atrial fibrillation	13 (12.03)	/
Angina pectoris	42 (38.9)	/
Previous myocardial infarction	21 (19.4)	/
CABG	3 (1.3)	/

CAGB – coronary artery bypass graft surgery; x̄– arithmetic mean; SD – standard deviation.

tachycardia, and second and third-degree atrioventricular block requiring a temporary or permanent pacemaker. Differences between ET-1 and NOx levels in patients with and without intrahospital complications are presented in Figures 2 and 3. A significant difference was found in ET-1 levels in patients with in-hospital echocardiographically detected complications (ventricular dyskinesia, left ventricular thrombus and papillary muscle rupture) compared to patients without those complications (Table 3). Similar results were not obtained regarding the NOx levels (Table 4). Higher incidence of intrahospital complications was associated with higher levels of ET-1 in a simple logistic analysis [OR = 1.440, 95% confidence interval (CI) (1.050–1.975); $p = 0.024$]. During 3 years, 29 (26.8%) patients were re-hospitalized due to cardiovascular causes and 13 (5.6%) patients died, of whom 3 (1.3%) died during the initial hospitalization. Except for one patient who died due to major gastrointestinal bleeding associated with dual antiplatelet therapy, all others died due to cardiovascular causes (re-infarction, sudden cardiac death and stroke). Survival curves for composite endpoint (3-year mortality and re-hospitalization) in patients with ET-1 and NOx concentrations below and above the 75th percentile are presented in Figure 4. Cox regression analysis showed that patients with ET-1 concentrations above the 75th percentile (≥ 3.79 pg/mL) had higher risk for mortality and/or rehospitalization during 3 years [hazard ratio (HR) = 2.893, 95%CI (1.595–5.246); $p < 0.001$]. A significant difference was not found between percentiles of NOx for a composite endpoint in Cox regression analysis.

Endothelin-1 was predictive for mortality in univariate analysis and kept independence in the multivariate logistic model adjusted for hsTnI, BNP, hs CRP, NOx, LVEF, gender, and type of AMI (STEMI *vs* NSTEMI). Though, ET-1 was predictor for 3-year mortality, as well as age (Table 5). In a simple logistic analysis, higher ET-1 levels [odds ratio (OR) = 1.398, 95%CI (1.054–1.854); $p = 0.02$] were associated with higher intrahospital mortality risk. Lower NOx levels [OR = 0.971, 95%CI (0.955–0.999); $p = 0.028$] and lower LVEF [OR = 0.959, 95%CI (0.918–0.999); $p = 0.037$] were associated with increased risk for re-hospitalization in a simple logistic analysis.

Interestingly, there were no significant differences in levels of ET-1 and NOx, among patients with STEMI and

Table 2

Laboratory parameters in study participants

Parameters	Experimental group	Control group	*p*
WBC (10^9/L), mean ± SD	11.25 ± 3.85	6.72 ± 1.35	$p < 0.001$
hs CRP (mg/L), median (IQR)	9.29 (4.52–35.65)	1.28 (0.56–2.81)	$p < 0.001$
CK-MB (U/L), median (IQR)	378.35 (161.45–715.57)	48.75 (21.90–97.80)	$p < 0.001$
hs TnI (ng/mL), median (IQR)	12.35 (3.02–41.27)	/	/
ET-1 (pg/mL), median (IQR)	3.08 (2.41–3.77)	3.04 (2.38–3.78)	n.s.
NOx (μmol/L), median (IQR)	131.80 (115.65–161.10)	89.3 (81.8–96.8)	$p < 0.001$

Data are presented as mean ± standard deviation or median with interquartile range (IQR) in parenthesis; WBC – white blood cell; CK-MB – creatine kinase MB isoenzyme; hs CRP- high sensitivity C-reactive protein; BNP – brain natriuretic peptide; hs TnI – high sensitivity troponin I; ET-1 – endothelin-1; NOx – nitric oxide degradation products (nitrates/nitrites).

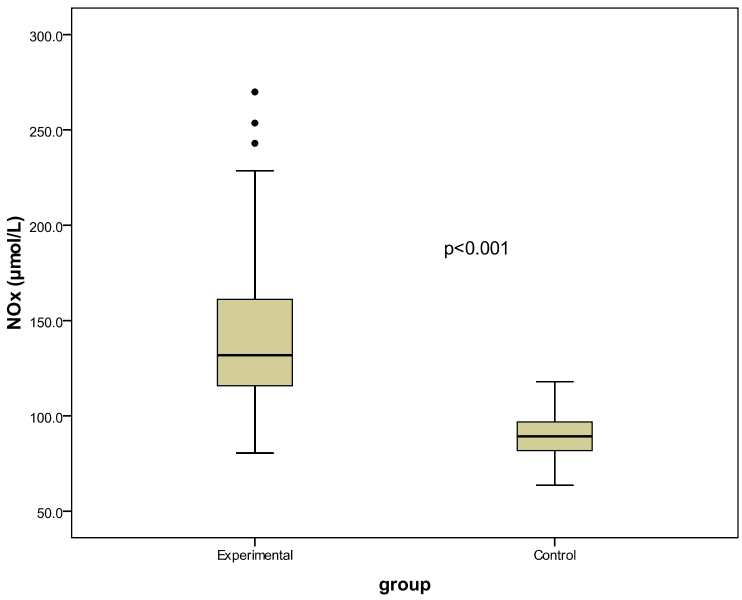

Fig. 1 – Nitric oxide degradation products (NOx) in the experimental and control groups. NOx are shown as median with interquartile range between 25th and 75th percentile. Values higher than 75th percentile are presented as dots.

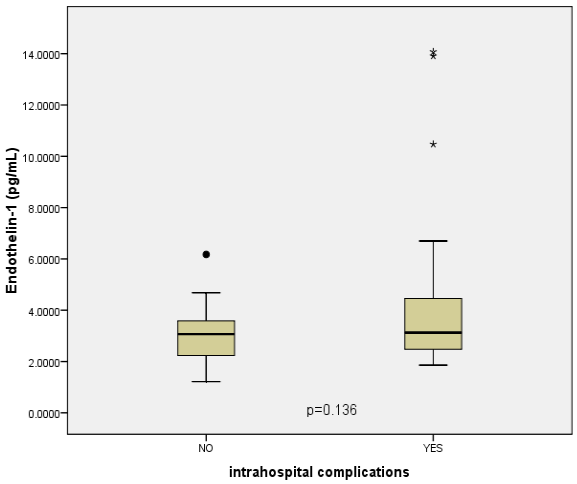

Fig. 2 – Endothelin-1 (ET-1) in patients with and without intrahospital complications (composite of arrhythmias and conduction disturbances, re-infarction, echocardiographycally detected complications and mortality). ET-1 concentrations are shown as median with interquartile range between 25th and 75th percentile. Values higher than 75th percentile are presented as dots and asterisks.

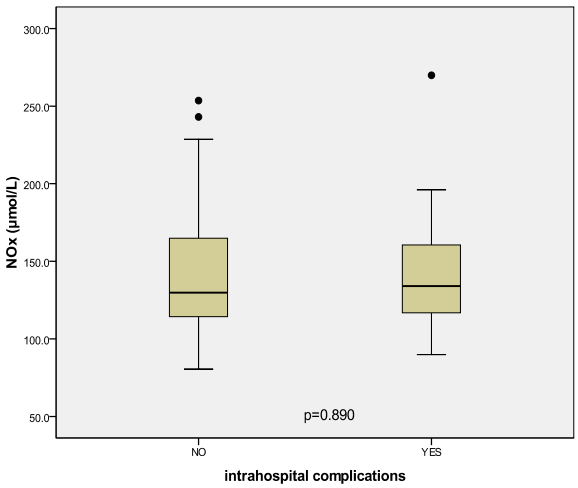

Fig. 3 – Nitric oxide degradation products (NOx) in patients with and without intrahospital complications (composite of arrhythmias and conduction disturbances, re-infarction, echocardiographycally detected complications and mortality). NOx concentrations are shown as median with interquartile range between 25th and 75th percentile. Values higher than 75th percentile are presented as dots and asterisks.

Table 3

Endothelin-1 (ET-1) levels in acute myocardial infarction patients with or without complications during 3 years

Complications	Patients n (%)	ET-1 level in patients without complication (pg/mL)	ET-1 level in patients with complication (pg/mL)	*p*
3-year mortality	13 (5.6)	3.06 (2.23–3.58)	4.02 (2.72–5.93)	0.015
In-hospital mortality	3 (1.3)	3.07 (2.34–3.71)	4.08 (3.28–8.99)	0.002
Arrhythmias and conductance disturbances	24 (22)	3.08 (2.47–3.72)	2.95 (2.21–4.11)	0.642
Re-infarction during initial hospitalization	5 (4.6)	3.08 (2.31–3.69)	4.45 (2.53–9.69)	0.160
Ventricular dyskinesia, ventricular thrombosis, papillary muscle rupture	9 (8.3)	3.06 (2.29–3.66)	4.02 (2.78–5.57)	0.012
Re-hospitalizations	29 (26.8)	3.06 (2.38–3.63)	3.11 (2.38–4.02)	0.585
Composite endpoint	46 (42.60)	3.05 (2.18–3.56)	3.14 (2.54–4.41)	0.035

Data are shown as median with interquartile range in parenthesis.

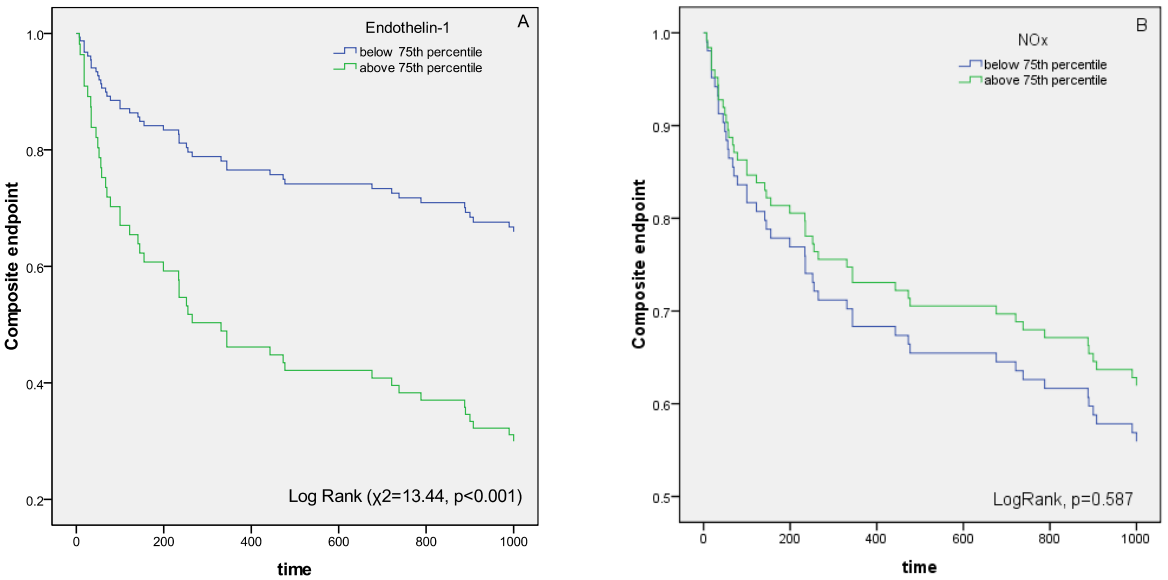

Fig. 4 – Kaplan-Meier curves for time until composite endpoint (mortality or rehospitalization) for ET-1 (A) and NOx (B) below and above 75th percentile.
NOx – Nitric oxide degradation products (nitrates/nitrites).

Table 4

Nitric oxide degradation products (NOx) in acute myocardial infarction patients with or without complications during 3 years

Complications	Patients n (%)	NOx levels in patients without complication (μmol/L)	NOx levels in patients with complication (μmol/L)	*p*
3-year mortality	13 (5.6)	131.8 (115.5–161.1)	139.3 (109.89–163.3)	0.962
In-hospital mortality	3 (1.3)	131.1 (114.9–160.8)	172.4 (155.85–178.65)	0.100
Arrhythmias and conductance disturbances	24 (22)	131.45 (114.6–164.275)	132.5 (116.8–158.775)	0.790
Re-infarction during initial hospitalization	5 (4.6)	131.8(116.1–161.1)	121.1 (105.35–175.2)	0.787
Ventricular dyskinesia, ventricular thrombosis, papillary muscle rupture	9 (8.3)	131.8 (115.5–161.1)	134.9 (113.95–170.2)	0.929
Re-hospitalizations	29 (26.8)	139.3 (116.79–165.2)	125.5 (111.4–143.6)	0.04
Composite endpoint	46 (42.6)	133.05 (115.2–164.9)	129.9 (115.44–160.5)	0.460

Data are shown as median with interquartile range in parenthesis.

Table 5

Logistic regression analyses for 3-year mortality

Variable	Simple model OR (95% CI)	*p*	Multiple model OR (95% CI)	*p*
Age	1,194 (1.022–1.272)	0.010	1.240 (1.047–1.468)	0.013
Male gender	0.761 (0.215–2.696)	0.761	not shown	ns
Ln ET-1	2.380 (1.073–5.774)	0.012	2.218 (1.171–4.202)	0.015
Ln NOx	0.997 (0.979–1.014)	0.692		ns
Ln TnI	1.006 (0.999–1.013)	0.119		ns
Ln hsCRP	0.873 (0.300–2.539)	0.803	not shown	ns
Ln BNP	3.378 (0.777–14.686)	0.105		ns
LVEF	0.988 (0.934–1.046)	0.682		ns
STEMI vs. NSTEMI	0.352 (0.107–1.156)	0.055		ns

STEMI – myocardial infarction with ST segment elevation; NSTEMI – myocardial infarction without ST segment elevation; LVEF – left ventricular ejection fraction; Ln – natural logarithm; OR – odds ratio; CI – confidence interval; ET-1 – endothelin-1; BNP – brain natriuretic peptide; hsCRP – high sensitivity C-reactive protein; NOx – nitric oxide degradation products (nitrates/nitrites); TnI – troponin; ns – non-significant.

NSTEMI [(ET-1: 3.14 (2.41–3.91) *vs* 2.76 (2.33–3.47) pg/mL; NOx: 131.80 (114.3–161.75) *vs* 131.45 (116.79–160.5) μmol/L], nor among those with STEMI treated with primary PCI or with thrombolytic therapy [(ET-1: 3.21 (2.41–3.98) *vs* 2.95 (2.31–3.68) pg/mL]; NOx: 128.6 (114.9–161.1) *vs* 165.5 (114.6–173.35) μmol/L].

Endothelin-1 positively correlated with hsTnI ($\rho = 0.291$; $p < 0.01$) and BNP levels ($\rho = 0.315$; $p < 0.05$) (Figure 5) and white blood cell (WBC) count ($\rho = 0.198$, $p < 0.05$).

Also, hsCRP correlated with hsTnI ($\rho = 0.399$; $p < 0.01$) and BNP levels ($\rho = 0.460$, $p < 0.01$). There were no correlations between NOx and other investigated biomarkers.

Discussion

Myocardial infarction continues to be a significant cause of mortality and morbidity in the western world. Biomarkers improve diagnostic and prognostic accuracy in AMI [16].

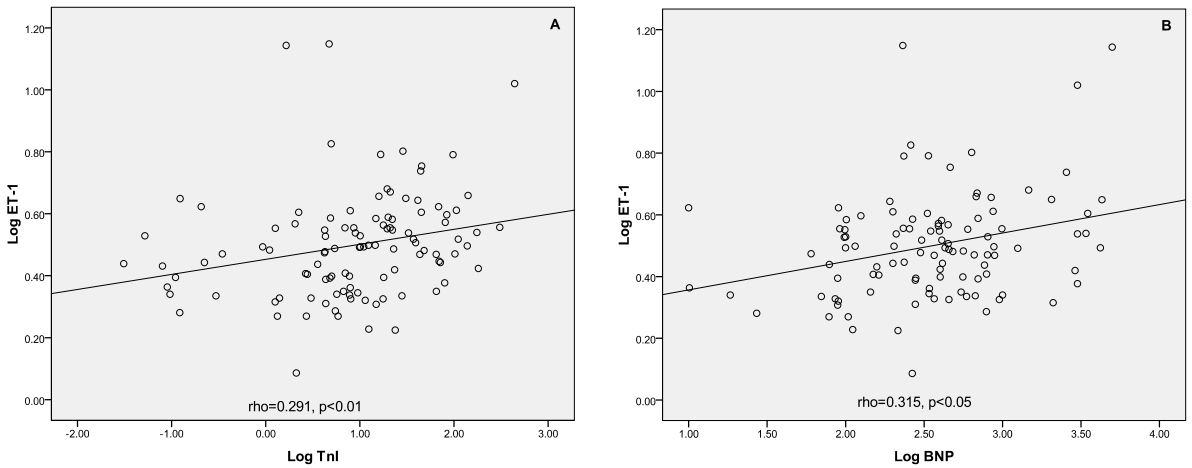

Fig. 5 – Linear correlation: A) between logarithmically transformed endothelin-1 (ET-1) and tropinin I (TnI) level values, and B) between logarithmically transformed ET-1 and brain natriuretic peptide (BNP) level values.

The clinical combination of hsCRP, troponins, and BNP has been used as a universal tool for risk stratification [17]. In our study, those biomarkers were not associated with the short or long-term cardiovascular mortality and morbidity. This could be the consequence of sample timing. Troponin I was measured only at admission. New studies emphasize the prognostic importance of TnI levels not only during the acute phase of NSTEMI, but also within the following 72 h. In large STEMI, the release of TnI over time is markedly different than in small NSTEMI or in micro-infarctions as a consequence of fluctuations in myocardial perfusion which has an impact on repeated measurements of TnI and its predictive role in different acute coronary syndromes [18, 19].

Endothelin-1 is synthesized in the vasculature and myocardium by various cell types [20]. Stewart et al. [21] showed that in uncomplicated AMI, ET-1 is elevated during a short period (reaches a peak after few hours), whereas in patients who develop heart failure, pulmonary oedema, shock or reinfarction, plasma ET-1 concentrations rise sharply and remain high during few days [21]. Therefore, to evaluate the relationship between plasma ET-1 and prognosis in AMI, it appears mandatory to measure plasma ET-1 after its early increase. That was the rationale for measurement of ET-1 on the 3rd day after AMI in the present study. Omland et al. [22] were first to describe a relation between ET-1 levels obtained in the subacute phase of AMI and 1-year mortality. In line with previous studies, our experimental group had higher levels of ET-1 than the control group, although insignificantly for those without complications. Also, elevated ET-1 levels were associated with higher risk for in-hospital and 3-year cardiovascular mortality. In particular, values above the 75th percentile were associated with a higher incidence of the composite endpoint (3-year mortality and rehospitalization).

In AMI patients with hemodynamic complications, the significant inverse relation was demonstrated between the highest plasma ET-l levels and LVEF [21]. Our patients with higher percentiles of ET-1 had the lowest LVEF, and we also found a significant positive relationship between ET-1 levels and BNP, which confirms the role of ET-1 in the hemodynamic complications, as an innocent bystander or involved in its pathogenesis, not known so far.

In experiments on animals, it was demonstrated that the rise of ET-1 has a significant impact on the size of the infarcted myocardial area. However, the correlation between ET-1 and creatine kinase MB Isoenzyme (CK-MB) was not found in previous studies [21, 23]. In the present study, ET-1 positively correlated with hsTnI. Accordingly, elevated ET-1 levels were associated with higher risk for intrahospital complications. Endothelin-1 was significantly higher in patients with echocardiographiocally detected complications (ventricular dyskinesia, left ventricular thrombus) and papillary muscle rupture compared to other AMI patients. Endothelin-1 is an arrhythmogenic substance, and that property is not associated with its other ability to induce myocardial ischemia in animal models [24]. However, we did not find a significant difference in ET-1 levels in patients with or without arrhythmias.

Endothelin-1 is a signaling molecule which transmits the signals to the leukocytes which are then activated and attracted to the myocardial tissue where they release reactive oxygen species (ROS) and cause injury of both myocardium and endothelium [25]. In our patients, ET-1 correlated with white blood cells (WBC) count.

Nitric oxide has a controversial role in AMI. Emerging evidence indicates that iNOS that produces NO is a hypoxia-inducible protein. Upregulation of iNOS is an important protective reaction of the heart in response to ischemia. Pre-existing endothelial (e)NOS is activated as a rapid response to myocardial ischemia but upregulation of iNOS leads to the continuous release of NO in this setting which is important for the protection of myocardial viability. Therefore, NO has a beneficial role in AMI [26]. It was shown that elevated ET-1 levels reduce NO bioavailability [27]. In our study however, ET-1 and NOx were not in correlation. It seems that in AMI activation of iNOS has a crucial role in regulation of NO production.

Experiments on animals demonstrated that after coronary artery occlusion NOx level is increased with a peak after 3 days. In patients with AMI, serial measurements of NOx after 24, 48, and 72 hours showed its significant increase compared to the control group. Nitric-oxide degradation products reached a peak after 2 or 3 days when iNOS was maximally stimulated by inflammatory cytokines [11]. This fact was the rationale for measurement of NOx on the 3rd day after AMI in the present study.

Accordingly, in our patients, we found an increase in NOx levels, which was more pronounced than ET-1 increase, on the 3rd day after AMI. Together with lower LVEF, lower NOx concentrations were predictors for re-hospitalization due to cardiovascular causes during 3 years. This could be the consequence of lower bioavailability of NO and endothelial dysfunction in extensive coronary artery diseases with a worse prognosis.

Mayyas et al. [28] found that ET-1 was significantly higher in patients with STEMI than in those with NSTEMI one week after an acute event. Reperfusion therapy in AMI increases oxidative stress which leads to decrease of NO and its protective effects during ischemia [29]. Eitel et al. [30] found that ET-1 measured prior to primary PCI was a marker of no-reflow and mortality in STEMI patients. Small sample studies showed long-term benefits of endothelin A receptor antagonists in STEMI patients given during primary PCI (less frequent no-reflow and higher LVEF). It seems that ET-1 is a prognostic indicator which is closely involved in the pathology of acute coronary syndrome and its complications [31, 32]. However, no difference was found in NOx and ET-1 levels between our patients with STEMI treated with a different type of reperfusion therapy, and those with NSTEMI. The timing of the assessment of biomarkers, with respect to the timing of the reperfusion therapy, could explain this finding.

Conclusion

Endothelin-1 and nitric-oxide degradation products were increased on the 3rd day after acute myocardial infarction, compared to healthy matching control. Endothelin-1 was significantly increased in patients with in-hospital papillary muscle rupture, echocardiographically detected complications (ven-

tricular dyskinesia, left ventricular thrombus) and in those who died during 3 years of follow-up. Endothelin-1 levels above the 75th percentile were associated with higher risk of 3-year mortality and rehospitalization. Decreased concentrations of nitric-oxide degradation products were associated with higher risk for rehospitalization during 3 years.

New biomarkers are emerging and it remains to be seen if consideration of endothelial dysfunction markers can add clinically important information to patient care. Compared to invasive or noninvasive imaging modalities, they offer the advantage of being relatively risk-free, less expensive, and applicable to a wide range of populations at risk.

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