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CORRELATIONS BETWEEN OXIDATIVE STRESS MARKERS AND CORONARY ANATOMY IN PERCUTANEOUSLY TREATED PATIENTS WITH ACUTE ST-ELEVATION MYOCARDIAL INFARCTION

Dan-Alexandru TĂTARU[a,b], Maria OLINIC[a,b*], Călin HOMORODEAN[a,b], Mihai-Claudiu OBER[b], Florin-Leontin LAZĂR[a], Andrada URDĂ-CÎMPEAN[a], Dan-Mircea OLINIC[a,b]

ABSTRACT. Understanding biochemical mechanisms involved in acute myocardial infarction is crucial for future treatments. The current study analyzed the oxidative stress markers in patients with percutaneously treated acute ST-elevation myocardial infarction and correlated the findings to coronary anatomy and Syntax II score (SS-II). Blood samples were obtained before coronary re-perfusion, after one and 24 hours. The following markers of oxidative stress were determined: malondialdehyde (MDA), reduced glutathione to oxidized glutathione ratio (GSH/GSSG) and total antioxidant capacity (TAC). Thirty-seven consecutive patients were included. The patients were divided into groups according to the infarct-related artery: left anterior descending artery (LAD-group) vs non-LAD group, and according to the calculated SS-II, SS-II≤ 34 vs. SS-II>34. MDA concentration and GSH/GSSG ratio showed non-significant differences between LAD vs non-LAD groups at all time frames. Patients with LAD as the infarct-related artery had a significantly lower TAC 24 hours after re-perfusion: 30.22 ± 9.78 % inhibition in the LAD group vs. 35.7 ± 5.78 % inhibition in the non-LAD group, p=0.013. The oxidative stress markers were similar between patients regardless of the SS-II value, and neither the culprit vessel nor the SS-II significantly influenced the dynamics of oxidative stress markers.

Keywords: *oxidative stress, myocardial infarction, Syntax Score.*

[a] *"Iuliu Haţieganu" University of Medicine and Pharmacy, Faculty of General Medicine, 8 Victor Babeş street, RO-400012, Cluj-Napoca, Romania.*
[b] *Cluj County Emergency Hospital, Interventional Cardiology Department, 3-5 Clinicilor street, RO-400006, Cluj-Napoca, Romania.*
[*] *Corresponding author: maria_olinic@yahoo.com*

INTRODUCTION

Cardiovascular diseases are the leading cause of death and illness worldwide [1]. Acute ST-elevation myocardial infarction (STEMI) occurs when a coronary artery is completely occluded, leading to myocardial necrosis. According to current evidence-based guidelines, primary percutaneous coronary intervention (pPCI) is the most effective method of opening the infarct-related artery [2]. However, sudden restoration of blood supply to an ischemic tissue creates the premises for reactive oxygen species (ROS) formation, which increases oxidative stress, leading to myocardial reperfusion injury (MRI) and microvascular obstruction (MVO) [3].

The mechanism involved in MRI has yet to be fully understood. Oxidative stress, lipid peroxidation, inflammation, MVO, and excessive catecholamine release are all responsible for the increase in infarct size [3].

Reactive oxygen species break down polyunsaturated lipids, producing malondialdehyde (MDA). This stable lipid peroxidation end-product is frequently used as a marker of oxidative stress [4].

Reduced glutathione (GSH) is an essential antioxidant and an electron donor, preventing damage to cellular components caused by ROS. Oxidized glutathione, also known as glutathione disulphide (GSSG), is a compound that gives information about the usage of GSH during oxidative stress reactions. In a resting cell, the molar GSH/GSSG ratio exceeds 100:1, while in various models of oxidative stress, this ratio could decrease to 10:1 and even 1:1 [5].

Total antioxidant capacity (TAC) is a measurement used to assess the antioxidant status of biological samples. It can evaluate the antioxidant response against the free radicals produced in a given disease [6].

The pathophysiology of acute myocardial infarction is very complex. Independent of the vessel involved, numerous biochemical pathways can severely influence post-infarction prognosis, leading to MRI and MVO. Evidence suggests that infarct size is not sufficient to explain differences in major adverse cardiac events (MACE) and mortality [7].

As the burden of coronary artery disease remains high after an acute coronary event, risk stratification is essential for effective treatment and reduction of mortality [8,9]. Myocardial infarction associated with the occlusion of the left anterior descending artery (LAD) is associated with an increased risk for MACE [10].

Thus, for adequate short- and long-term risk stratification in post-STEMI patients, the Syntax-II Score (SS-II) was validated, independent of other factors influencing post-infarction mortality [11]. The SS-II integrates, in addition to anatomic coronary characteristics, relevant clinical variables such as age, gender, left ventricular ejection fraction (LVEF), renal impairment, chronic obstructive pulmonary disease (COPD) and peripheral artery disease (PAD).

According to current evidence, the SS-II is a faithful predictor of MACE and mortality in STEMI patients treated by pPCI. A SS-II>34 cut-off is associated with the highest rate of MACE and the highest short-term cardiovascular mortality [12].

This work aimed to identify correlations between the infarct-related artery, the SS-II, and oxidative stress markers in patients with STEMI treated by pPCI.

RESULTS AND DISCUSSION

In the present study, 37 consecutive patients were included for statistical analysis.

The culprit vessels were the LAD in 18 patients (LAD group), the circumflex artery (Cx) in 10 patients and the right coronary artery (RCA) in 9 patients (Cx and RCA (19 patients) - non-LAD group). The clinical characteristics of the groups are presented in Table 1. Hospital stay was significantly longer in the LAD group (p=0.003).

Table 1. Clinical characteristics of the LAD and non-LAD groups; LAD - left anterior descending artery; SD - standard deviation; LVEF - left ventricular ejection fraction.

Group name	LAD group	non-LAD group
Age (years), mean ± SD	64.7 ± 11.1	62.2 ± 12.4
Male patients (%)	55	73
Total ischemia time (hours), mean ± SD	6.22 ± 3.29	6.47 ± 4.01
LVEF (%), mean ± SD	44.44 ± 7.25	46.66 ± 6.85
Hospital stay (days), mean ± SD	6.44 ± 4.23	3.47 ± 1.21

In the LAD group, the mean MDA was 2.9 ± 0.86 nmol/ml at P0 vs. 2.67 ± 0.85 nmol/ml, at P1 (p=0.098) vs. 2.18 ± 0.94 nmol/ml at P24 (p=0.002). At P0, the median value of the GSH/GSSG ratio was 3.30 (2.02-4.77) vs. 2.18 (1.3-2.98) at P1 (p=0.02) vs. 1.98 (1.49-2.75) at P24 (p=0.004). TAC had no significant variations from baseline, p=0.334.

In the non-LAD group, the mean MDA was 3.28 ± 1.04 nmol/ml at P0 vs. 2.68 ± 0.78 nmol/ml at P1 (p<0.001) vs. 2.12 ± 0.96 nmol/ml at P24 (p<0.001). The GSH/GSSG ratio was 2.94 (2.18-5.35) at P0 vs. 2.34 (1.88-2.98) at P1, p=0.002 vs. 1.79 (1.25-3.12) at P24, p=0.001. TAC had no significant variations, p=0.437.

Table 2 depicts the values of the oxidative stress parameters according to the culprit vessel.

Table 2. Oxidative stress markers at P0, P1 and P24 between the LAD and non-LAD groups (Mann-Whitney U test); P0 - baseline before reperfusion; P1 - one hour after reperfusion: P24 - 24 hours after reperfusion; LAD - left anterior descending artery; MDA - malondialdehyde; GSH - reduced glutathione; GSSG - oxidized glutathione; TAC - total antioxidant capacity; Normally distributed data is presented as mean ± standard deviation; Data without normal distribution is presented as median (interquartile range).

Group name	**LAD**	**non-LAD**	
			p-value
P0			
MDA (nmol/ml)	2.9 ± 0.86	3.28 ± 1.04	0.214
GSH (µmol/ml)	4.9 (3.04-6.67)	4.91 (3.53-6.3)	0.967
GSSG (µmol/ml)	1.82 (1.03-2.33)	1.58 (1.22-2.49)	0.928
GSH / GSSG	3.30 (2.02-4.77)	2.94 (2.18-5.35)	0.919
TAC (inhibition %)	31.64 ± 5.22	33.69 ± 4.39	0.157
P1			
MDA (nmol/ml)	2.67 ± 0,85	2.68 ± 0.78	0.976
GSH (µmol/ml)	4.25 (2.58-7.51)	3.96 (2.99-6.55)	0.714
GSSG (µmol/ml)	1.97 (1.69-2.34)	2 (1.5-2.74)	0.932
GSH / GSSG	2.18 (1.3-2.98)	2.34 (1.88-2.98)	0.529
TAC (inhibition %)	29.97 ± 7.11	34.07 ± 5.10	**0.019**
P24			
MDA (nmol/ml)	2.18 ± 0.94	2.12 ± 0.96	0.854
GSH (µmol/ml)	3.81 (2.98-5.62)	4.91 (4.04-6.41)	0.102
GSSG (µmol/ml)	2.15 (1.64-2.51)	2.7 (1.72-4.24)	0.136
GSH / GSSG	1.98 (1.49-2.75)	1.79 (1.25-3.12)	0.601
TAC (inhibition %)	30.22 ± 9.78	35.7 ± 5.78	**0.013**

Only TAC had different dynamics between the two groups: P0, 31.64 ± 5.22 % of inhibition in the LAD group vs. 33.69 ± 4.39 % of inhibition in the non-LAD group, p=0.154; P1, 29.97 ± 7.11 % inhibition in the LAD group vs.

34.07 ± 5.10 % of inhibition in the non-LAD group, p=0.019; P24, 30.22 ± 9.78 % inhibition in the LAD group vs. 35.7 ± 5.78 % inhibition in the non-LAD group, p=0.013.

For all 37 patients, the median value of SS-II was 28.1 (20.65-41.1). The clinical characteristics of the SS-II≤34 and SS-II>34 are presented in Table 3. In the SS-II>34 group LVEF was notably lower (p=0.058) and hospital stay was significantly longer (p=0.005), compared to the SS-II≤34 group.

Table 3. Clinical characteristics of the SS-II≤34 and SS-II>34 groups; SD - standard deviation; LAD - left anterior descending artery; LVEF - left ventricular ejection fraction.

Syntax-II Score	**≤34**	**>34**
Number of patients	23	14
Age (years), mean ± SD	58.3 ± 8.5	72.1 ± 9.8
Male patients (%)	87	28.5
LAD infarct-related artery (%)	34.8	71.5
Total ischemia time (hours), mean ± SD	6.26 ± 3.67	6.51 ± 3.71
LVEF (%), mean ± SD	47.8 ± 3.3	41.7 ± 8.4
Hospital stay (days), mean ± SD	3.56 ± 1.23	7.14 ± 4.53

Table 4 shows the values of the oxidative stress markers between the two SS-II groups. The SS-II did not influence the dynamics of the markers determined in this study.

In the SS-II≤34 group, MDA had significant variations: P0, 3.21 ± 1.08 nmol/ml; P1, 2.77 ± 0.79 nmol/ml, p=0.001; P24), 2.27 ±0.96 nmol/ml, p<0.001. Also, the GSH/GSSG ratio had significant changes: P0, 3.1 (2.18–5.02; P1, 2.02 (1.54-2.62), p<0.001; P24; 1.98 (1.49-2.75), p<0.001. TAC had no significant variations in this group of patients.

In the SS-II>34 group, MDA decreased significantly after re-perfusion: P0, 2.91 ± 0.72 nmol/ml; P1, 2.52 ± 0.83 nmol/ml, p=0.032; P24, 1.93 ± 0.91 nmol/ml, p=0.004. Moreover, GSH/GSSG ratio decreased significantly: P0, 3.69 (2.02-6.00); P1, 2.48 (1.35-4.10), p=0.074; P24, 2.3 (1.43-3.25), p=0.011. TAC had no significant changes.

The present study aimed to establish a connection between oxidative stress markers and coronary anatomy in STEMI patients. Both short-term and long-term prognoses are more severe for patients with STEMI and multi-vessel coronary artery disease compared to patients with single-vessel disease

[13]. Also, significant evidence suggests that RCA occlusion is linked to better post-infarction survival, whereas LAD occlusion is associated with the highest mortality after STEMI [14]. This study showed that the LAD was the main vessel responsible for infarction (almost three-quarters of patients) in patients with high SS-II. Other authors have reported similar results [14].

Table 4. Oxidative stress markers at P0, P1 and P24 between the SS-II≤34 and SS-II>34 groups (Mann-Whitney U test); P0 - baseline before reperfusion; P1 - one hour after reperfusion: P24 - 24 hours after reperfusion; LAD - left anterior descending artery; MDA - malondialdehyde; GSH - reduced glutathione; GSSG - oxidized glutathione; TAC - total antioxidant capacity; Normally distributed data is presented as mean ± standard deviation; Data without normal distribution is presented as median (interquartile range).

Syntax-II Score	**≤34**	**>34**	
			p-value
P0			
MDA (nmol/ml)	3.21 ± 1.08	2.91 ± 0.72	0.352
GSH (µmol/ml)	4.95 (3.47-7.6)	4.58 (3.64-6.67)	0.715
GSSG (µmol/ml)	1.94 (1.22-2.58)	1.55 (0.8-2.09)	0.164
GSH / GSSG	3.1 (2.18-5.02)	3.69 (2.02-6.00)	0.528
TAC (inhibition %)	31.66 ± 4.64	34.39 ± 4.88	0.119
P1			
MDA (nmol/ml)	2.77 ± 0.79	2.52 ± 0.83	0.312
GSH (µmol/ml)	4.95 (2.77-7.3)	3.98 (2.68-7.72)	0.937
GSSG (µmol/ml)	2 (1.5-3.09)	1.97 (1.5-2.32)	0.484
GSH / GSSG	2.02 (1.54-2.62)	2.48 (1.35-4.10)	0.411
TAC (inhibition %)	31.94 ± 5.32	32.30 ± 8.13	0.824
P24			
MDA (nmol/ml)	2.27 ± 0.96	1.93 ± 0.90	0.283
GSH (µmol/ml)	4.91 (3.73-5.5)	3.89 (3.05-7.1)	0.503
GSSG (µmol/ml)	2.66 (1.74-3.34)	2.15 (1.5-2.45)	0.274
GSH / GSSG	1.98 (1.49-2.75)	1.79 (1.25-3.12)	0.368
TAC (inhibition %)	32.97 ± 6.85	33.14 ± 10.63	0.821

Regardless of the time frame, MDA, GSH, GSSG, and the GSH/GSSG ratio exhibited similar values in the LAD and non-LAD groups. TAC had comparable values at baseline but was significantly lower in the LAD group after reperfusion. This fact suggests increased oxidative stress and a depletion of the antioxidative mechanism in the LAD group. The larger myocardial mass involved in LAD-related myocardial infarction can lead to the formation of numerous reactive oxygen species on the first day after reperfusion [15,16]. In addition to acute heart failure, mortality is related to life-incompatible malignant arrhythmias. Evidence shows that LAD involvement predisposes to ventricular arrhythmias [17], and increased oxidative stress can induce these devastating phenomena [18]. A low TAC value indicates a large amount of reactive oxygen species and could explain the molecular mechanisms that more frequently induce life-threatening arrhythmias in LAD-related myocardial infarction. These observations may explain the higher mortality present in patients suffering from an anterior STEMI.

Evidence shows that vascular territories not involved in infarction also suffer from acute ischemia through mechanisms such as MVO or arteriolar spasm [19]. However, no significant differences were noticed between the oxidative stress markers in patients from the SS-II≤34 vs. SS-II>34 groups. It seems that co-morbidities such as COPD, PAD and renal impairment do not have a direct impact on short-term oxidative stress dynamics.

Several published works have shown a significant decrease in MDA after pPCI in the clinical context of STEMI treated by pPCI [15,16,20]. In this study, MDA decreased significantly in all patient subgroups, and this change was not influenced by the infarct-related artery or by the severity of the coronary disease indicated by the SS-II. Moreover, in the present paper, the GSH/GSSG ratio decreased significantly in all patient groups. Other studies with similar protocols have also reported similar results [15,21].

TAC did not vary significantly in the first 24 hours, regardless of the culprit vessel or SS-II. Literature data suggest TAC has specific dynamics after coronary re-perfusion [15]. Although simple to perform, the 2,2-diphenyl-1-picrylhydrazy (DPPH) method has the drawback of non-linear response to plasma volume. This facet points to the need for careful comparisons of results obtained under non-identical conditions. However, it does not invalidate the possibility of monitoring the trend in changes of TAC within one protocol [22].

The results of this study suggest that the production of free radicals, in the context of STEMI, depends not only on the myocardium involved but also on the body's ability to counter and control the production of reactive oxygen species.

This study's major limitations include the small number of patients and the early loss of follow-up, which prevented data on MACE from being obtained.

CONCLUSIONS

This study found that patients with LAD-related STEMI had a significantly lower TAC 24 hours after reperfusion than patients with STEMI due to another artery occlusion.

The specific culprit vessel did not influence plasma levels of MDA or the GSH/GSSG ratio. Additionally, the study determined that the oxidative stress markers were similar between patients, regardless of the calculated SS-II.

Neither the culprit vessel nor the SS-II significantly influenced the dynamics of oxidative stress markers.

EXPERIMENTAL SECTION

The research was conducted following the Declaration of Helsinki [23]. Written consent was obtained from each patient before the procedure. Consecutive STEMI patients treated by pPCI were included in the study. The inclusion criteria were as follows: electrocardiographic evidence of ST elevation of ≥ 1 mV in two or more standard limb leads or ≥ 2 mV in two or more precordial leads; typical chest pain lasting more than 20 minutes; presentation within 12 hours since symptom onset; successful pPCI of the culprit vessel.

Two physicians with experience in interventional cardiology analyzed the angiographic images for each patient and independently calculated the SS-II using the algorithm available online at https://syntaxscore.org [11]. The patients were divided into groups as follows: patients with the LAD as the infarct-related artery (LAD group) vs. patients with the Cx or the RCA as the culprit (non-LAD group); patients with low SS-II≤34 vs. high SS-II>34.

Peripheral venous blood samples were obtained from each patient immediately before pPCI (P0), one hour after reperfusion (P1), and after 24 hours (P24). Blood samples were collected into plastic tubes with ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1500 rpm for 15 minutes. The resulting plasma was stored at –30°C until analysis. The following oxidative stress markers were determined at each harvest: MDA, GSH, GSSG, GSH/GSSG ratio and TAC. The dynamics of these markers were compared according to the previously mentioned patient groups.

MDA was measured using the method outlined by Conti et al. [24]. Initially, 50 µl of plasma was boiled with 1 mL of 10 mmol/L 1,3-diethylthiobarbituric acid (DETBA) reagent in phosphate buffer (0.1 mol/L, pH =3). After a 60-minute incubation, the resulting DETBA-MDA product was extracted using 5 ml of n-butanol. The solution was centrifuged at 1500 rpm for 15 minutes. The fluorescence of the supernatant was then assessed at an emission wavelength of 534 nm using a spectrofluorometer (Lambda 35, Perkin Elmer, USA) with a synchronous fluorescence technique at a 14 nm difference between the excitation and emission wavelengths ($\Delta\lambda$). MDA concentration was determined using a standard solution incorporating different MDA concentrations (62.5 to 250 pmol of MDA per 50 µl), employing the same measurement technique. The MDA levels were reported in nmol/ml.

GSH was determined after a method proposed by Hu [25]. First, 500 µl of plasma was mixed with 500 µl of cold 10% trichloroacetic acid (TCA). After 10 minutes in ice, the mixture was centrifuged at 3000 rpm for 15 minutes. After that, 200 µl of the supernatant was mixed with 1.7 ml of sodium phosphate 0.1 M / EDTA 5 mM buffer, pH=8 and 0.1 ml of o-phthalaldehyde (1 mg/ml in absolute methanol). Using a spectrofluorometer (Lambda 35, Perkin Elmer, USA), the fluorescence at 350 nm excitation and 420 nm emission was read against a blank that contained deionized water to replace plasma.

GSSG was estimated using Vats' method [26]. Initially, 250 µl of the plasma sample was incubated with 0.1 ml of 40 nM N-ethylmaleimide for 30 minutes, followed by an addition of 0.65 ml of 0.1 M NaOH. After that, the same procedure was followed for fluorescence development as in GSH measurement, except 0.1 M NaOH was put instead of the buffer. The GSH and GSSG values were calculated from standard curves and were expressed in µmol/ml.

TAC was determined according to Janaszewska et al. [22]. The reduction assay was performed by adding 20 µl of plasma to 400 µl of 0.1 mM methanol solution of DPPH and a phosphate buffer, pH=7.4. After a 30-minute incubation at ambient temperature, the absorbance of the samples at 520 nm was measured with a spectrofluorometer (Lambda 35, Perkin Elmer, USA) and compared with that of a control sample containing only DPPH and a phosphate buffer. TAC was measured in inhibition per cent (%) as [(control extinction - serum extinction) / control extinction] x 100.

All the reagents supplied by Sigma (Deisenhofen, Germany) were of analytical grade and were used without further purification. All the water used was doubly distilled.

The statistical analysis was conducted using SPSS software v25 (IBM, USA). The Shapiro-Wilk test was used to assess for normal distribution [27]. Quantitative data without normal distribution was described using box plots,

median and Q1-Q3, where Q1-Q3 (interquartile range) stands for the range between the 25th percentile (Q1) and the 75th percentile (Q3). Normally distributed data was presented as mean ± standard deviation.

The non-parametric Mann-Whitney U test was used to compare samples from unpaired samples [28], and the Wilcoxon Signed Ranks test was used to analyze oxidative stress parameters within the same group [29]. For normally distributed samples, the paired samples t-test was used [28]. The threshold of statistical significance was set at $p \leq 0.05$.

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