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Arylesterase activity of Paraoxonase 1 - prognostic factor for one-year survival in patients with acute myocardial infarction

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Abstract

***Introduction**: Reduced serum levels of paraoxonase 1 (PON1) activities are associated with diseases involving increased oxidative stress, such as acute coronary syndrome. We aimed to determine whether serum PON1 activities are a prognostic factor for one-year survival following ST-elevation myocardial infarction (STEMI).*

***Material and methods**: We prospectively followed for one-year 75 patients diagnosed and treated for STEMI. Clinical, laboratory and imagistic data were gathered after coronary angiography. PON1 activities (paraoxonase, arylesterase, and lactonase) were assayed spectophotometrically on samples of heparinized plasma taken from the patients in a timeframe of maximum 20 minutes after coronary angiography.*

***Results**: Increased mortality was linked to age (patients over 68 years), permanent atrial fibrillation or left ventricular ejection fraction (LVEF) <40% (associated with global hypokinesia, apical or septal akinesia), trivascular disease atherosclerosis, reduced PON1 activities (paraoxonase <18.4 IU/mL, arylesterase <12.6 IU/mL, lactonase <27.6 IU/mL), and glomerular filtration rate levels <54 mL/min/1.73m2. Multivariate survival analysis*

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showed the independent prognostic role of age (HR 3.92; 95%CI 1.08-14.16; p=0.03), LVEF (HR 9.93; 95%CI 2.20-44.86; p=0.003) and arylesterase (HR 4.25; 95%CI 0.94-19.18; p=0.05) for one-year mortality.

***Conclusion**: Reduced arylesterase activity of PON1 is an independent predictor of one-year survival after acute myocardial infarction.*

***Keywords**:paraoxonase, arylesterase, lactonase, ST-elevation myocardial infarction, survival*

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Introduction

Paraoxonase 1 (PON1) is an enzyme with lactonase/esterase properties, encoded by a gene located on chromosome 7q21.3 [1,2] and associated with high-density lipoproteins (HDLs) containing apolipoprotein J (clusterin) [3]. Once associated, the PON1-HDL complex can hydrolyze a wide range of substrates (organophosphorus compounds, paraoxone, unsaturated aliphatic esters or aromatic carboxylic acids) [2,4]. PON1 activities display interindividual variations and low levels have been directly associated with the initiation and progression of atherosclerosis [1]. Correct evaluation of PON1 activity requires the concomitant determination of all three activities of PON1, as recent studies have demonstrated that its native activity is lactonase [5].

ST-elevation myocardial infarction, a consequence of atherosclerosis, is one of the most common causes of cardiovascular death [6]. Many studies have demonstrated that oxidative stress and inflammation play an important role in atherosclerotic plaque destabilization and the occurrence of acute myocardial infarction [7]. Reduced values of PON1 activities have been associated with diseases involving increased oxidative stress [8,9], such as acute coronary syndrome [10].

Multiple factors influence medium and long-term prognosis of patients with acute myocardial infarction, including: history of myocardial infarction, age over 75 years, severity of coronary lesions, interventional or surgical revascularization procedures, chronic heart disease, obesity, pharmacological treatment or the presence of comorbidities (diabetes mellitus, renal disease) [11].

Recent study data are inconsistent regarding PON1 activity and survival rates following acute myocardial infarction, partially explainable because PON1 activities exhibit inter-individual and inter-ethnic variations, largely due to PON1 gene polymorphisms [12-15]. On the other hand, in a certain population, even among individuals with the same genotype, PON1 activity may show variations of 13 up to 40 times. This can be explained by the multitude of exogenous factors, lifestyle, age or different physiological or pathological states that can influence PON1 levels [1,16].

The objective of this study was to determine whether PON1 activity is a prognostic factor for one-year survival following acute ST-elevation myocardial infarction.

Material and methods

The STARD consensus for reporting diagnostic/prognostic studies was followed [17]. We conducted a prospective, observational study screening a consecutive series of 270 patients who presented for acute coronary syndrome (unstable angina, non-ST-Elevation myocardial infarction - NSTEMI and ST-Elevation myocardial infarction - STEMI) at the Cardiology Department of the Clinical Emergency County Hospital from Braşov, Romania, between March 2013 and March 2014.

Inclusion criteria were as follows: patients diagnosed with STEMI and treated by angioplasty in the Intensive Coronary Unit of the same hospital and the patients did not satisfy any of the exclusion criteria.

Exclusion criteria were: informed consent not given, inability to understand the given explanations about the study or hemodynamic instability, death, unstable angina, NSTEMI, medical history of STEMI and any other conditions that might have influenced the serum PON1 activities (acute infection, neoplasia, hepatic cirrhosis, chronic obstructive pulmonary disease stage GOLD IV, thyroid gland dysfunctions, psychiatric disorders, severe chronic kidney disease (CKD) with an estimated creatinine clearance <30 ml/minute/m^2, and alcohol consumption in the last 6 months more than 20 g/day for men and 10 g/day for women).

The STARD diagram which shows the selection of our study population is showed in figure 1.

A total of 75 patients were included in the study. Mean age in the study group was 64 (55; 75) years, and the male to female ratio was 2:1 (50 men and 25 women). Using the criteria in place [18,19], the STEMI diagnosis was established. All patients underwent coronary angiography. All patients had signed the informed consent for the inclusion in the study. The study protocol was approved by the Ethics Committee

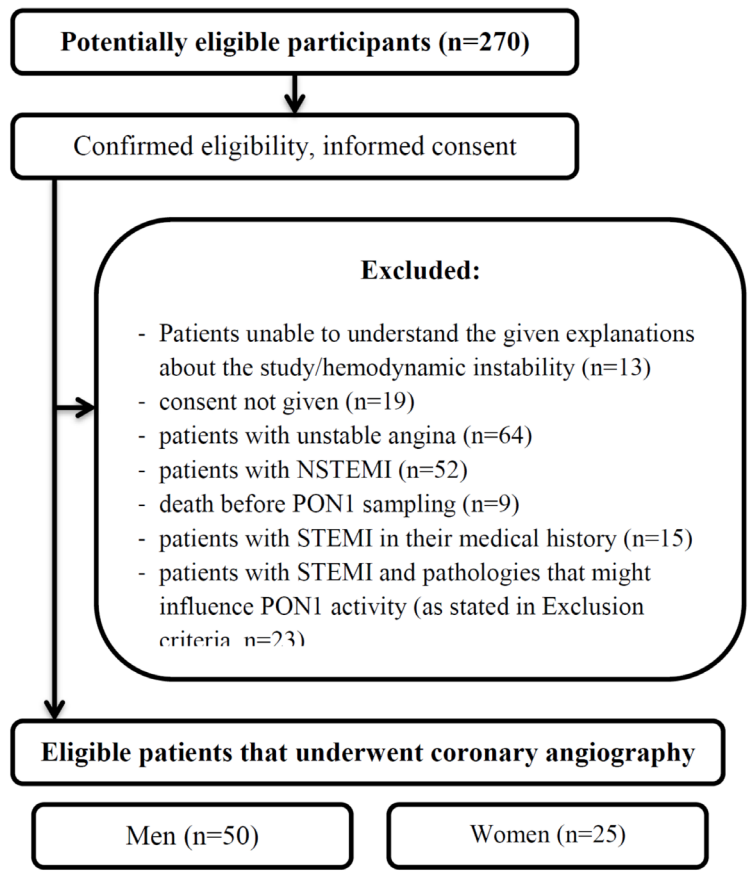

Figure 1 The STARD diagram for the selection of the study population

of Transilvania University from Brasov, according to the ethical guidelines of 1975 Declaration of Helsinki. For the purpose of consistency, all included patients received aspirin, clopidogrel, beta-blockers and statins. Clinical data recorded on admission were age, gender, smoking history, hypertension, dyslipidemia, diabetes mellitus, previous myocardial infarction, chronic coronary disease or rhythm disorders. Grace score was calculated, which is a robust tool of predicting the six-month mortality in acute coronary syndromes [20], using an online calculator [21]. Body mass index (BMI) was also measured. Left ventricular ejection fraction (LVEF) was estimated according to Simpson`s method [22] along with segmental or global myocardial hypokinesia using an ALOKA Prosound SSD-4000SV echocardiograph (Hitachi-Aloka Ltd, Tokyo, Japan).

Laboratory data collected were high-density cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides, urea, creatinine, uric acid, highly specific C reactive protein (hsCRP), CK, CK-MB, troponin T. Laboratory testing was performed using the Roche COBAS 6000 analyzer (diagnostic division of Hoffmann-La Roche AG, Basel, Switzerland) from the Central Laboratory of the Clinical County Emergency Hospital in Brasov. eGFR was calculated using the Modification in Diet in Renal Disease equation (MDRD) [23]. Patients were followed up to 12 months following acute STEMI, and the date of death was documented.

PON1 activities were determined by spectophotometric methods from heparinized plasma obtained after angioplasty, using the protocol first described by Eckerson in 1983 [24], with minor modifications. Paraoxon (O, O-diethyl-Op-nitrophenylphosphate) was used to measure paraoxonase activity. Arylesterase and lactonase activities were measured using phenylacetate and control samples were used to make corrections in case of spontaneous decomposition of the substrate activity [25,26]. Duplicate samples were used and the resulting data were expressed in U/l for paraoxonase activity and kU/l for PON1 arylesterase and lactonase activities. Paraoxonase 1 enzymatic activity determinations were performed in the Medical Biochemistry Department of the 3rd Molecular Sciences Department within "Iuliu Haţieganu" University of Medicine and Pharmacy in Cluj-Napoca. The determinations used blood harvested immediately after performing myocardial revascularization.

Statistical analysis was performed using MedCalc Statistical Software version 17.5.5 [27]. Quantitative data were tested for normality of distribution with Kolmogorov-Smirnov test. Quantitative data were expressed by median and 25-75 percentiles and qualitative data were chracterized by absolute and relative frequency. Differences between groups were verified using chi-square test or Mann-Whitney test, as appropriate. Analysis of survival was carried out using Cox regression models. Cut-off values were calculated by AUROC (Area Under Receiver Operating Characteristic). A p value <0.05 was considered statistically significant.

Results

Patient demographic data and comorbidities are presented in Table 1. One-year mortality rate was 18.6%. Deceased patients were of significantly higher age, were diagnosed with permanent atrial fibrillation and had a lower BMI.

Patients' echocardiographic and coronarographic data are presented in Table 2. Low LVEF, global hypokinesia, septal or apical akinesia and trivascular atherosclerosis were associated with mortality.

Laboratory data are presented in Table 3. Lactonase, arylesterase, hemoglobin, renal function were the variables associated with increased mortality risk.

Table 1. Demographic data and comorbidities of patients with acute myocardial infarction

Variable		Survivors (61)	Deceased (14)	P
Age (median, 25-75 percentiles)		60 (52.5; 73)	78.5 (66.7; 84.5)	<0.001
Sex	Women	19 (31.1%)	6 (42.9%)	0.5
	Men	42 (68.9%)	8 (57.1%)	
BMI (body mass index) (kg/m^2) (median, 25-75 percentiles)		30 (26; 32.4)	25.5 (22; 28)	0.02
Smokers		39 (63.9%)	7 (50%)	0.5
History of AMI (acute myocardial infarction)		7 (11.5%)	4 (28.6%)	0.2
Permanent atrial fibrillation		4 (6.6%)	6 (42.9%)	0.002
Diabetes mellitus		34 (55.7%)	8 (57.1%)	1
Hypertension		41 (67.2%)	10 (71.4%)	1
History of CKD (chronic kidney disease) creatinine clearance >30 ml/min/m^2		20 (32.8%)	6 (42.9%)	0.5
Dyslipidemia		17 (27.9%)	4 (28.6%)	1
ACE (angiotensin-converting-enzyme) inhibitors/ ARBs (angiotensin II receptor blockers)		49 (89.3%)	10 (71.4)	0.4
Oral anticoagulant		4 (6.6%)	5 (35.7%)	0.1
Grace risk score (median, 25-75 percentiles)		104 (88.5; 125)	163.5 (131.7; 197.5)	<0.001

Table 2. Echocardiographic and coronarographic data associated with mortality

Variable	Survivors (61)	Deceased (14)	P
LVEF (left ventricular ejection fraction) (median, 25-75 percentiles)	45 (42.5; 50)	32.5 (25; 40)	<0.001
Global hypokinesia	4 (6.6%)	7 (50%)	<0.001
Septal hypokinesia	24(39.3%)	2 (14.3%)	0.1
Anterior hypokinesia	10 (16.4%)	2 (14.2%)	1
Lateral wall hypokinesia	12 (19.7%)	3 (21.4%)	1
Apical hypokinesia	23 (37.7%)	2 (14.3%)	0.1
Septal akinesia	5 (8.2%)	4 (28.6%)	0.05
Apical akinesia	3 (4.9%)	6 (42.9%)	<0.001
Coronary thrombus aspiration	5 (8.2%)	-	0.5
Percutaneous transluminal coronary angioplasty	35 (57.4%)	4 (28.6%)	0.07
ADA (anterior descending artery) stent	23 (37.7%)	3 (21.4%)	0.3
ACX (circumflex artery) stent	7 (11.5%)	-	0.3
ACD (right coronary artery) stent	9 (14.8%)	1 (7.1%)	0.6
Trivascular coronary atherosclerosis	12 (19.7%)	7 (50%)	0.03

ROC curves were used in order to calculate cut-off values for several variables for better differentiating between deceased and survivors (table 4).

To determine the role of the independent prognostic factor of the studied variables we built several models. Given the high degree of correlation between the various variables and the small number of patients in the study, the final model included age >68 years, LVEF <40% and arylesterase <12.6 IU/mL (Table 5).

Discussion

In the current study, the value of PON1 activities and other clinical and biochemical parameters as prognostic factors for one-year survival following STEMI were evaluated. Numerous clinical trials have demonstrated that the risk of mortality in patients with acute coronary syndromes gets higher with age. Moreover, age is a parameter used in C-ACS and GRACE scores that assess cardiovascular risk in patients with

Table 3. Laboratory data associated with mortality

Variable	Survivors (61)	Deceased (14)	P
Paraoxonase (IU/mL) (median, 25-75 percentiles)	26.6 (18.9; 57.3)	14.8 (11; 43)	0.1
Arylesterase (IU/mL) (median, 25-75 percentiles)	13.8 (10.8; 16.7)	10.7 (8; 12)	0.006
Lactonase (IU/mL) (median, 25-75 percentiles)	26.6 (21; 34.3)	20.6 (14.7; 25.6)	0.009
hsCRP (High-sensitivity C-reactive protein) (nmol/L) (median, 25-75 percentiles)	23 (8; 43)	47.6 (10.5; 56.1)	0.1
Hemoglobin (g/dL) (median, 25-75 percentiles)	13.5 (12; 15)	13 (11.5; 13)	0.04
Uric acid (mg/dL) (median, 25-75 percentiles)	6 (5; 7.2)	5 (4; 7)	0.1
Urea (mg/dL) (median, 25-75 percentiles)	40 (34.2; 50.7)	75 (36.5; 122)	0.005
Creatinine (mg/dL) (median, 25-75 percentiles)	1 (0.8; 1.1)	1.3 (1; 1.5)	0.003
eGFR (Estimated glomerular filtration rate) (mL/min/1.73m^2) (median, 25-75 percentiles)	74 (58.8; 91.1)	51.6 (32.7; 68.8)	0.001
Total cholesterol (mg/dL) (median, 25-75 percentiles)	197 (166; 225)	181 (153; 217)	0.2
HDL cholesterol (mg/dL) (median, 25-75 percentiles)	46 (41; 49)	44 (41; 52)	0.5
LDL cholesterol (mg/dL) (median, 25-75 percentiles)	100 (89; 117)	99.5 (77.25; 108.2)	0.4
Triglycerides (mg/dL) (median, 25-75 percentiles)	148 (108.5; 190.5)	179.5 (71; 200.2)	0.7

Table 4. ROC curve analysis

Characteristic	AUC		Sensitivity %		Specificity %		p
	value	CI 95%	value	CI 95%	value	CI 95%	
Age > 68 years	0.801	0.693-0.884	78.57	42.9-95.3	70.49	57.4-81.5	< 0.001
LVEF* < 40%	0.820	0.715-0.899	85.71	57.2-98.2	75.41	62.7-85.5	< 0.001
Paraoxonase <18.4 IU/mL	0.629	0.509-0.738	57.14	28.9-82.3	77.5	64.5-86.6	0.1
Arylesterase < 12.6 IU/mL	0.736	0.621-0.831	92.86	66.1-99.8	55.74	42.8-68.5	< 0.001
Lactonase < 27.6 IU/mL	0.726	0.611-0.823	92.86	66.1-99.8	47.54	34.6-60.7	< 0.001
eGFR < 54 ml/min/1.73m2	0.785	0.674-0.871	71.43	41.9-91.6	83.61	71.9-91.8	< 0.001

*Normal LVEF is considered > 50%

Table 5. Multivariate analysis for survival

Variable	B	P	HR	95.0% CI for HR	
				Min	Max
Age >68 years	1.36	0.03	3.92	1.08	14.16
LVEF <40%	2.29	0.003	9.93	2.20	44.86
Arylesterase <12.6 IU/mL	1.44	0.05	4.25	0.94	19.18

acute coronary syndromes [20,28]. We noticed that elderly patients had a high risk of cardiovascular mortality due to acute myocardial infarction, especially over 68 years. This is consistent with other studies in which advanced age appears to affect patients` mortality, mainly due to recurrence of myocardial infarction [29].

Among echocardiographic and coronarographic features, we noticed that LVEF, global hypokinesia, akinesia of the apex and trivascular lesions were statistically associated with the highest death rate one year following STEMI. Low LVEF and global hypokinesia were associated with higher death rates following STEMI in other studies [30]. In our study, an association between low hemoglobin levels and increased mortality was found, anemia being a well-known aggravating factor for myocardial ischemia [31].

Paraoxonase 1 is a complex enzyme, capable to catalyze the hydrolysis of multiple substrates, such as organophophates, lactones, hydroperoxides, and arylesters. Furthermore, it associates with HDL particles containing clusterin and protects them from transformation into dysfunctional HDL, together with inhancing its activities of removing the excess of lipids from the walls of the arteries (a key anti-atherogenic mechanism of HDL-PON1 complex) [32]. As stated before, the natural activity of the enzyme is lactonase [26], used to remove lactone-like substrates such as oxidized low-density lipoproteins, which are known to be a major cause of inflammation and propagation of atherosclerosis. Using its arylesterase activity, PON1 is also capable of hydrolysing aromatic carboxylic acid esters, thus reducing the oxidative stress even more [1, 32].

Although there were different statistically significant values between paraoxonase, arylesterase, and lactonase activities between the two groups (survivors and deceased) and knowing that PON1 activity is closely related to HDL levels, we had also expected to find these differences in HDL levels, which we did not find in our study. This could be explained by the fact that PON1 only associates with HDL3 particles in the entire HDL spectrum, namely lipid-poor and containing apolipoprotein J [3]. Apolipoprotein J is essential for both PON1 transfer from hepatocytes to HDL and modulation of PON1 activity [2,4]. In the current study, low arylesterase, lactonase, and paraoxonase levels were associated with increased one-year mortality following infarction, data consistent with other research data [32]. Szpakowicz *et al*. found that the subjects carrying *PON1* gene polymorphisms are at a higher risk of death after STEMI [33]. Although in the univariate analysis all three PON1 activities were statistically significantly associated with increased one-year mortality risk following STEMI, multivariate analysis only emphasized arylesterase activity as an individual prognostic factor. It has been shown that there is a reduction in PON1 activities with ageing, most likely due to the alteration of sulfhydryl groups. Arylesterase activity is the only one not influenced by age. Our findings show that the other PON1 activities (paraoxonase, lactonase) lost the value of independent risk factors, most likely due to the fact that they are influenced by age [1], which in our study was an independent prognostic factor.

PON1 activities have a protective role against peroxidized lipoproteins that accumulate on

LDL-cholesterol and induce progression of atherosclerosis [35]. The increased level of PON1 activities was associated with better survival in patients with coronary artery bypass grafting surgery [36]. The patients who died in our study had low PON1 activities. The etiology of myocardial infarction in our study was atherosclerosis, which is known to be promoted by high levels of oxidative stress. Given the fact that PON1 is an antioxidant enzyme which protects against the development of atherosclerosis, it is clear why low levels of PON1 activities correlate with advanced atherosclerosis and thus, consequently, with lower survival rates following an acute myocardial infarction. There were no significant differences between hsCRP levels in deceased vs. alive patients in our study (Table 3). This is consistent with other studies which have proven that hsCRP has a prognostic value immediately after an acute coronary syndrome, but it is not an independent predictor of long-term survival after such acute cardiovascular events [37].

Our study has several limitations: the small number of participants included due to the very restrictive exclusion criteria needed to eliminate the factors that might influence PON1 levels and the relatively short follow-up time following STEMI (one year). We acknowledge that in-hospital mortality was not pre-defined as a clinical outcome, because it could have been related to the comorbidities of the patients and that would have required a much bigger study group with many patterns of patients in order to retrieve a relevant result. Also, we acknowledge that we have not been able to collect with accuracy the correct ischemic period between symptom onset and revascularization therapy. This was due to a variety of issues related to the patients (e.g. diabetic patients without any thoracic pain) or related to the ambulance service.

Conclusion

Reduced PON1 arylesterase activity, reduced LVEF, and advanced age of subjects with STEMI are independent prognostic factors for one-year mortality following myocardial infarction.

Author's contribution

LC concept, methodology, writing, editing, supervision; MG concept, methodology, investigation, editing, supervision; ŞCV formal analysis, resources, data curation, editing, supervision; AT resources, review and editing; GD resources, visualization; TA investigation, visualization; MM draft preparation, review, editing, visualization, AM investigation, resources, data cupation; GS investigation, resources, writing, supervision; IP conceptualization, resources, writing, visualization, supervision.

LC and MG had equal contribution to the paper.

Conflict of interests

None to declare

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