MEDICAL UNIVERSITY - PLEVEN

DEPARTMENT OF CARDIOLOGY, PULMOLOGY AND
ENDOCRINOLOGY

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Analysis of clinical and laboratory factors in the 9-year survival of patients with acute coronary syndrome

DOCTORAL DISSERTATION

for the acquisition of the scientific and educational degree

Doctor

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Contents

USED ABBREVIATIONS:

ACE-inhibitor - Inhibitor of Angiotensin-Converting Enzyme

AP, ABP – Arterial Blood Pressure

ARB - Angiotensin Receptor Blocker

AH – Arterial Hypertension

SMC – Smooth Muscle Cells

DBP - Diastolic Arterial Pressure

RBBB - Right Bundle Branch Block

ED - Endothelial Dysfunction

ECG- Electrocardiography

EchoCG - Echocardiography

DM – Diabetes Mellitus

IHD - Ischemic Heart Disease

IS – Ischemic Stroke

CAD – Coronary Artery Disease

CK – Creatine Kinase

CK/MB - Creatine Kinase-MB

LBBB – Left Bundle Branch Block

LPHB - Left Posterior Hemiblock

LVH - Left Ventricular Hypertrophy

LAHB - Left Anterior Hemiblock

CVD – Cerebrovascular Disease

UAP – Unstable Angina Pectoris

Non-HDL-C - Non-High Density Lipoprotein Cholesterol

AMI – Acute Myocardial Infarction

PAD - Peripheral Arterial Disease

PCI - Percutaneous Coronary Intervention

AF – Atrial Fibrillation

PAMI - Previous Acute Myocardial Infarction

CA antagonist - Calcium Channel Antagonist

CBP - Systolic Blood Pressure

SAP – Stable Angina Pectoris

HF – Heart Failure

WHO - World Health Organization

SCA – Selective Coronary Angiography

CVD - Cardiovascular Disease

CVR - Cardiovascular Risk

CVE – Cardiovascular Event

TG - Triglycerides

TIA - Transient Ischemic Attack

CKD - Chronic Kidney Disease

CRF – Chronic Renal Failure

USED LATIN ABBREVIATIONS

BMI - body mass index

BP - blood pressure

CAD - coronary artery disease

ESC - European Society of cardiology

GRACE - Global registry of acute coronary events score

HDL-C - High -density lipoprotein

LDL-C - Low-density lipoprotein

NSTEMI - non-ST-elevation myocardial infarct

PON 1 - paraoxonase 1 enzyme

SCORE - Systematic Coronary Risk Evaluation

STEMI - ST-elevation myocardial infarct

TG - Triglycerides

TVD- triple-vessel disease

UA - unstable angina

1. INTRODUCTION

The data from clinical studies and registries for risk stratification after hospital discharge are scarce. In Bulgaria, there are a limited number of interventional studies involving patients with ACS, which provide insights into the prognosis and survival of patients after hospital discharge. There is a need to determine the risk profile of this heterogeneous group of patients and obtain data on their short-term and long-term survival and mortality rates.

Determining the prevalence of modifiable and non-modifiable risk factors among hospitalized individuals with ACS, confirming the traditional risk scales for short-term prognosis following ACS, searching, identifying, and incorporating additional prognostic factors could be of value for long-term prognosis of patients. Including additional variables to the traditional risk factors and searching for new ones among the Bulgarian patient population, in particular healthy control subjects, could contribute to the early detection of high-risk groups and timely implementation of primary and secondary prevention and treatment measures.

2. GOAL AND OBJECTIVES

2.1. GOAL:

Investigation of some clinical, instrumental, biochemical, and certain genetic markers that form the risk profile of patients with ACS and analyze their contribution to the short-term and long-term prognosis of this group of patients.

2.2. OBJECTIVES:

1. To perform a demographic distribution of patients with ACS and determine the frequency of non-modifiable and modifiable risk factors, as well as assess the contribution of the factor of time from the onset of clinical symptoms.

2. To analyze the relationship of the biochemical parameters (TC, LDL, HDL, TG, creatinine, CPK, CPK-MB, Tn I) with the demographic and risk factors.

3. To analyze some echocardiographic parameters such as left ventricular ejection fraction (LVEF) and its relationship with short-term and long-term prognosis of patients with ACS.

4. To analyze the relationship between the morphological changes identified by invasive examination and the known risk factors.

5. To calculate the GRACE-score and its association with all-cause mortality during hospital treatment on 6th month, 1st, 3rd, 5th, and 9th year.

6. To determine the serum paraoxonase and arylesterase activity of PON1, distribution and frequency of the tested polymorphic variant L55M PON1 in patients with ACS and heathy control subjects, and to analyze the relationship of the abovementioned with biochemical parameters and survival rate.

7. To develop a prognosis model in patients with ACS for 1st, 5th, and 9th year survival rate.

3. MATERIALS AND METHODS.

3.1. STUDY SUBJECTS:

Patients admitted as emergencies to the Cardiology Clinic of UMBAL-Stara Zagora and SBALC-Yambol were included at random in the conducted study during the period from January 2009 to February 2010. The local Ethics Committee of Trakia University in Stara Zagora approved the inclusion of patients in the dissertation.

In the study are included consequently 172 patients with ACS and the distribution by diagnosis is the following: STEMI - 103 (59.88%), NSTEMI - 25 (14.53%), UA - 44 (25.59%). The average total age of the entire group is 61.5±11.9(32-86). The distribution by gender is the following: women-59 (34.3%), men-113(65.7%) at average age respectively for women 64.5±11.2 (42-85) and men - 60.0±12.0 (32-86). All patients have been emergently admitted to the Intensive Care Unit (ICU) of the Cardiology Department with clinical, electrocardiographic, and biochemical markers for ischemia, and biochemical tests were performed on them.

Inclusion criteria:

➢ Over 18 years of age
➢ Oral consent for participation in the study and patient follow-up
➢ Signing informed consent for collection of biological material reflected in the medical history

➤ Criteria meeting the diagnostic algorithm for STEMI, NSTEMI, UA according to the European guidelines

Exclusion criteria:

➤ Under 18 years of age
➤ Lack of consent from the individual to participate in the study

3.2. METHODS:

3.2.1. Documentary methods;

3.2.2. Clinical methods;

3.2.3. Laboratory methods

Biochemical indicators: examination of the levels of the total serum cholesterol, LDL-C, HDL-C, TT, blood sugar, urea, creatine, CPK, CPK-MB, Tn I, PON-activity, ArEs-activity, L55M PON1 (the last three indicators were examined only in a limited number of patients due to financial constraints within the allocated additional funds from a won research project from MU Stara Zagora);

3.2.4. Genetic methods

Genotyping regarding the polymorphism PON1L55M (rs 854560);

3.2.5. Instrumental methods:

ECG, echocardiography, selective coronary angiography (SCA);

3.2.6. Statistical methods:

As level of significance at which the null hypothesis is rejected $p < 0.05$ was adopted. The results are presented not only as text but also through a series of tables, graphs, and numerical values (percentages, coefficients, means, etc.).

- Description of the quantitative variables;
- Graphical method;
- Analysis of variance;
- Parametric and nonparametric analysis;
- Correlation analysis;
- Regression analysis;
- Other statistical methods – ROC-analysis;

4. RESULTS

4.1. Demographic distribution within the groups and frequency of some non-modifiable and modifiable risk factors in ACS. Determining the contribution of the time factor from the onset of the clinical symptomatic.

172 patients with ACS have been included within the study and the distribution by diagnosis is the following: STEMI - 103, NSTEMI - 25, UA - 44. All patients were admitted urgently to the Intensive Care Unit (ICU) of the Cardiology department with clinical, electrocardiographic, and biochemical indications for ischemia and biochemical tests have been made on them.

The average age of patients diagnosed with STEMI is 60.9±12.1 (32.0;85.0). The men are four years younger than the women, and the group of men is twice as large as that of women. On table 1 is represented the demographical distribution of patients with AMI with ST-elevation. The demographic characteristic of the patients with ACS without ST-elevation is shown on Table 1.

Таблица 1. Demographic characteristic of the group of patients with STEMI, NSTEMI and UA.

Demographic indicators	STEMI (n=103)	NSTEMI (n=25)	UA (n=44)	p-value
Gender number (%) Women Men	37(35.9) 66(64.1)	14(31.8) 30(68.2)	8(32.0) 17(68.0)	0.851
Age (years) mean±SD (range)				0.093
Total:	60.9±12.1(32.0;85.0)	66.2±12.3(42.0;86.0)	60.2±10.7(39.0;86.0)	
Women	63.6±11.1(42.0;85.0)	69.9±15.3(42.0;82.0)	63.7±8.6(49.0;76.0)	
Men	59.4±12.5(32.0;83.0)	64.5±10.6(48.0;86.0)	58.6±11.3(39.0;86.0)	

The distribution of non-modifiable and some modifiable risk factors is shown in Table 2.

Risk factors	STEMI (n=103) number (%)	NSTEMI (n=25) number (%)	UA (n=44) number (%)	p-value

Suffered ACS	20(19.4)	13(52.0)	17(38.6)	0.002
Family history	25(24.3)	6(24.0)	12(27.3)	0.921
Smoking smokers ex-smokers non-smokers did not specify	 37(35.9) 5(4.9) 58(56.3) 3(2.9)	 6(24.0) 5(20.0) 14(56.0)	 19(43.2) 5(11.4) 20(45.5)	0.092
Dyslipidemia	60 (58.3)	28(63.6)	19(76)	0.253
Arterial hypertension Total Women Men	 85(82.5) 34(91.9) 51(77.3)	 21(84.0) 7(87.5) 14(82.4)	 39(88.6) 13(92.9) 26(86.7)	 0.647 0.902 0.548
Diabetes mellitus (known and newly discovered) NIDDM[1] IDDM[2] IGT[3]	 18(17.5) 6(5.8) 5(4.9)	 4(16.0) 2(8.0) -	 11(25.0) 4(9.1) 1 (4.0)	0.697
BMI** average±SD (range) median (range)	30.0±4.3(19.0-55.4) 26.5(19.0-55.4)	28.3±4.3(21.5;41.9)	27.0±3.3(19.0;33.8)	0.301
Glomerular filtration (ml/min) average±SD (range) Total Women Men	 69.15±27.3(4.8;158.8) 60.9±25.7(4.8;108.6) 73.7±27.3(11.4;158.8)	 68.37±23.3(28.2;125.0) 62.9±31.3(28.2-125.0) 70.9±19.1(40.6-105.5)	 78.45±16.5(52.8;116.6) 62.3±10.6(52.8-91.5) 83.7±16.2(57.9-116.6)	 0.089 0.701 0.109

Table 3. Distribution of risk factors and biochemical tests by gender.

Risk factors	Women	Men	Significance
Average age	64.5±11.2(42.0-85.0)	60.0±12.0(32.0-86.0)	p<0.01
AH	54	91	p=0.06
Smoking	14	48	p<0.001

[1] Non-insuline dependent diabetes mellitus
[2] Insuline dependent diabetes mellitus
[3] Impaired glucose tolerance

|			
Dyslipidemia	36	71	p=0.81
Diabetes mellitus	14	31	p=0.6
Family history	14	29	p=0.78
CAH (mmHg) median (range)	140.0(105.0; 260.0)	132.0(90.0; 240.0)	p=0.6
DBP (mmHg) median (range)	80.0(60.0; 143.0)	80.0(60.0; 130.0)	p=0.77
HR (bpm) median (range)	81,8±17,6	77.5±15,1	p=0.045
TC (mmol/l) median (range)	5.4(3.0; 14.3)	5.2(1.4; 16.1)	p=0.203
LDL (mmol/l) median (range)	3.1(1.2; 10.8)	2.9(0.7; 12.1	p=0.217
HDL (mmol/l) median (range)	1.7(0.6; 3.3)	1.7(0.8; 2.8	p=0.573
TG (mmol/l) median (range)	1.2(0.4; 3.3)	1.0(0.3; 5.8))	p=0.527
Creatinine(µmol/l) average±SD (range)	115.0±133.6 (50.0;815.7)	103.2±46.3 (61.2; 460.2)	p=0.116
GFR (ml/min) average±SD	62.7±23. 6	76.0±24. 0	p=0.001

From the data in Table 3 above, it is evident that at the male gender, ACS manifests on average about 4.2 years earlier than in females, regardless of the diagnosis (p=0.09). There is a tendency in the non-ST-elevation ACS group to have individuals of advanced age (p=0.097).

For studying the relationships between the age and the examined indicators we divided the patients into three age groups: <=49 years, 50-69 years and >=70 years. We discovered marginal in significance correlation between the age and the frequency of occurrence of diabetes mellitus: the highest frequency of diabetes occurs in patients in the age group over 70 years compared to the younger age groups (p=0.055) (Figure 1).
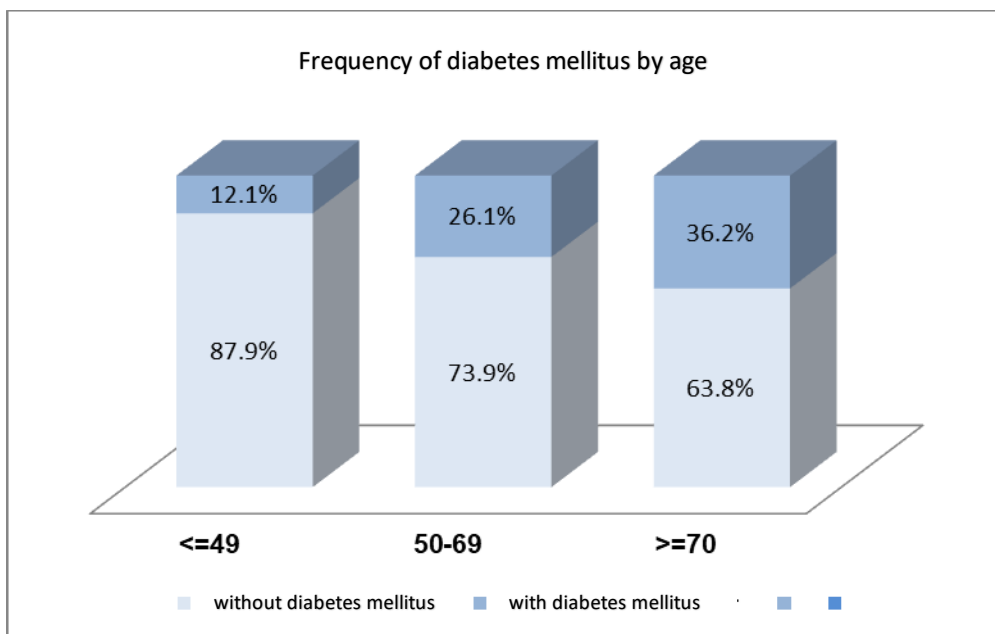
Figure 1. Distribution of the occurrence of diabetes mellitus by age.
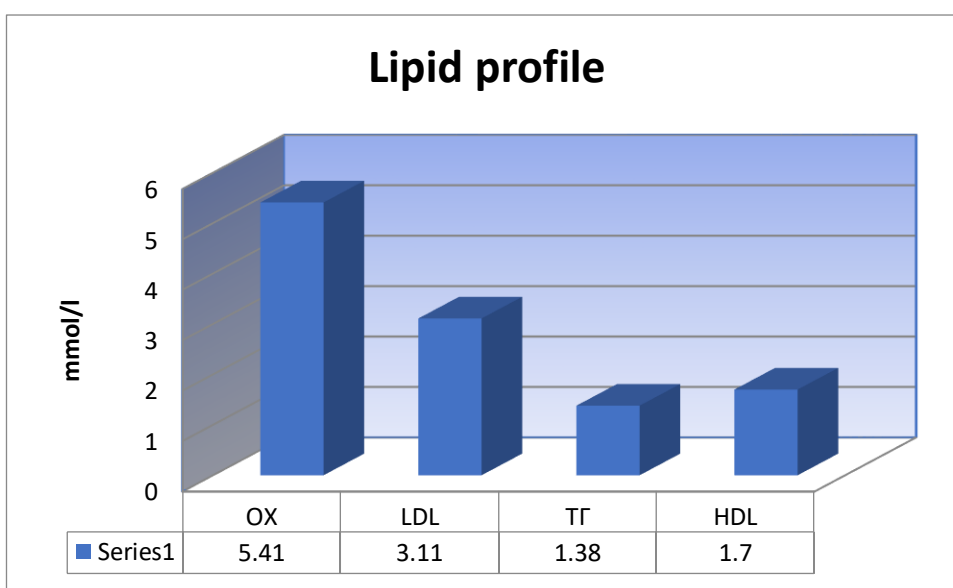
Figure 2. Lipid profile in general group

Table 4 presents the demographic characteristics, some risk factors, clinical and instrumental data of patients in the three groups. Table 4A, 4B, 4C, and 4D present the demographic characteristics, medical history, clinical, instrumental, laboratory, and biochemical data in the entire study group.

Таблица 4A: Demographic, medical history and documentational data of the patients in the entire group.

	Total number(%)	STEMI Number(%)	NSTEMI Number(%)	UA Number(%)
Number of patients	172	**103(59.9%)**	**25(14.5%)**	**44(25.6%)**
Gender (% men)	113(65.6)	66(64.1%)	17(68.0%)	30(68.2%)
Age (years) mean±SD (range)	61.5±11.9 (32.0-86.0)	60.9±12.1 (32.0-85.0)	66.2±12.3 (42.0-86.0)	60.2±10.7 (39.0-86.0)
Risk factors				
without RF 1 RF (% yes) 2 RF (% yes) >3 RF (% yes)	4(2.3%) 15(8.7%) 47(27.3%) 106(61.6%)	4(3.9%) 9(8.7%) 29(28.2%) 61(59.2%)	0(0%) 2(8.0%) 8(32.0%) 15(60.0%)	0(0%) 4(9.1%) 10(22.7%) 30(68.2%)
Known heart failure>II F.C.				
	11(6.4%)	6(5.8%)	3(12.0%)	2(4.5%)
Previous stable angina				
	86(50.0%)	39(37.9%)	15(60.0%)	32(72.7%)
Anemia	19(11.1%)	14(13.6%)	2(8.0)	3(6.8%)
CVD	14(8.3%)	9(8.7%)	1(4.0)	4(9.1%)
Onset of ischemic pain				
<6 hours 6-12 hours 13-24 hours >24 hours	39(22.9%) 17(10.0%) 18(10.6%) 96(55.8%)	31(30.4%) 13(12.7%) 13(12.7%) 45(43.2%)	3(12.5%) 4(16.7%) 5(20.8%) 12(50.0%)	5(11.4%) - - 39(88.6%)
Outpatient treatment:				
ACE/Total ARB Beta-blockers Statin Aspirin Clopidogrel Trimetazidine Loop diuretics	81(47.1%) 65(37.8%) 31(18.0%) 48(27.9%) 11(6.4%) 9(5.2%) 10(5.8%)	34(33.0%) 22(21.4%) 7(6.8%) 17(16.5%) 4(3.9%) 5(4.9%) 4(3.9%)	18(72.0%) 12(48.0%) 9(36.0%) 12(48.0%) 4(16.0%) 2(8.0%) 4(16.0%)	29(65.9%) 31(70.5%) 15(34.1%) 19(43.2%) 3(6.8%) 2(4.5%) 2(4.5%)

Table 4B: Laboratory and clinical data of the studied group.

Laboratory indicators: mean±SD(range)			
TC (mmol/l)	5.4±1.7 (1.4; 16.1)	5.6±1.9 (1.4; 16.1)	5.3±1.6 (3.0-11.1)	5.0±1.0 (3.0-7.8)
LDL (mmol/l)	3.1±1.4 (0.7; 12.1)	3.3±1.6 (1.2; 12.1)	3.0±1.4 (0.7-8.0)	2.8±0.9 (1.2-5.0)
HDL (mmol/l)	1.7±0.4	1.8±0.4	1.6±0.3	1.6±0.4

	(0.7; 3.3)	(0.8; 3.3)	(1.2; 2.5)	(0.7-2.8)
TG (mmol/l)	1.4±0.9 (0.3; 5.8)	1.3±0.9 (0.3; 5.8)	1.6±1.0 (0.6; 3.7)	1.4±0.9 (0.3; 3.8)
Creatinine (μmol/l)	107.2±85.8 (50.0; 815.7)	117.1±107.8 (54.2; 815.7)	101.1±30.5 (50.0; 163.5)	86.4±12.7 (63.0; 124.2)
CPK (U/l)	723.9±1179.4 (25.0; 6491.0)	1011.5±1405.5 (64.0; 6491.0)	506.9±575.7 (25.0; 1827.0)	161.2±297.1 (34.0; 2020.0)
CPK-MB (U/l)	103.2±161.2 (1.2; 1108.1)	143.5±191.3 (2.5; 1108.1)	74.4±86.4 (2.7; 270.7)	25.5±42.8 (1.2; 251.2)
TnI (pg/ml)	6.9±27.8 (0.01; 226.0)	8.5±34.6 (0.01; 226.0)	5.4±8.2 (0.09; 28.1)	2.6±3.2 (0.1; 9.6)
SBP (mmHg) mean±SD (range)	137.4±26.8 (70.0; 260.0)	138.9±29.7 (70.0; 260.0)	141.8±26.4 (100.0; 210.0)	131.4±18.0 (80.0; 170.0)
DBP (mmHg mean±SD (range)	85.0±15.4 (40.0; 143.0)	86.0±17.2 (40.0; 143.0)	85.8±14.2 (60.0; 118.0)	82.3±10.8 (50.0; 106.0)
HR (bpm) mean±SD (range)	79.0±16.1 (40.0; 145.0)	80.2±16.3 (40.0; 135.0)	78.6±12.9 (59.0; 104.0)	76.3±17.1 (50.0; 145.0)
ALVHF: KillipII-IV	27(15.7%)	14(13.6%)	2(8.0%)	11(25.0%)

Table 4B: Instrumental data of the studied group.

Echocardiographic indicators mean±SD (range)				
LVDD	50.3±7.5 (24.0; 80.0)	50.7±7.4 (31.0; 80.0)	48.5±8.1 (24.0; 66.0)	50.3±7.4 (39.0; 71.0)
LVSD	34.0±7.8 (21.0; 60.0)	35.0±7.4 (21.0; 55.0)	35.4±8.1 (23.0; 55.0)	34.6±8.6 (23.0; 60.0)
EF n(%)				
>55-50%	96(55.8%)	49(47.6%)	12(48.0)	35(79.5%)
49-40%	55(32.0%)	39(37.9%)	11(44.0)	5(11.4%)
39-30%	9(5.2%)	6(5.8%)	1(4.0)	2(4.5%)
<30%	9(5.2%)	6(5.8%)	1(4.0)	2(4.5%)
Left atrium	36.2±7.7 (12.7; 57.0)	35.9±8.3 (12.7; 57.0)	35.2±7.1 (24.0; 52.0)	37.6±6.7 (21.0; 52.0)
Mitral insufficiency n(%)				
I	48(27.9%)	22(21.4%)	6(24.0%)	20(45.5%)
II	18(10.5%)	11(10.7%)	4(16.0%)	3(6.8%)

III	6(3.5%)	5(4.9%)	1(4.0%)	-
ECG				
ST-elevation	96(55.8%)	90(87.4%)	4(16.0%)	2(4.5%)
ST-depression	31(18.0%)	8(7.8%)	13(52.0%)	10(22.7%)
Neg. T-wave	39(22.7%)	9(8.7%)	9(36.0%)	21(47.7%)
Patolog. Q-wave	51(29.7%)	35(34.0%)	6(24.0%)	10(22.7%)
Without ST-T-changes	5(2.9%)	0(0.0%)	1(4.0%)	4(9.1%)
Cardiac rhythm disorders:				
LBBB	4(2.3%)	1(1.0%)	1(4.0%)	2(4.5%)
RBBB	8(4.7%)	3(2.9%)	4(16.0%)	1(2.3%)
LAHB.LPHB	17(9.9%)	9(8.7%)	3(12.0%)	5(11.4%)
AV-block	13(7.6%)	10(9.7%)	1(4.0%)	2(4.5%)
Atrial fibrillation/flutter	19(11.0%)	10(9.7%)	4(9.1%)	5(20.0%)
Ventricular tachycardia	7(4.1%)	6(5.8%)	1(2.3%)	-
Coronary anatomy				
n	156	101	25	30
LM	18(11.5%)	9(8.9%)	4(16.0%)	5(16.7%)
LAD	96(61.5%)	62(61.4%)	17(68.0%)	17(56.7%)
LCx	64(41.3%)	34(33.7%)	17(68.0%)	13(44.8%)
RCA	88(56.8%)	57(56.4%)	18(72.0%)	13(44.8%)
Single-vessel disease	51(32.7%)	40(39.6%)	4(16.0%)	7(23.3%)
Double-vessel disease	46(29.5%)	33(32.7%)	7(28.0%)	6(20.0%)
Triple-vessel disease	48(30.0%)	26(25.7%)	13(52.0%)	9(30.0%)
Without CD	11(7.1%)	2(2.0%)	1(4.0%)	8(26.7%)
Referred for CABG	14(8.1%)	10(9.7%)	-	4(9.1%)

Table 4 D: Data from the in-hospital mortality, PON/ArEs-activity (U/L) and GRACE-score in the studied group.

In-hospital mortality	13(7.6%)	11(10.7%)	-	2(4.5%)
GRACE				
<=108	90(52.3%)	51(49.5%)	8(32.0%)	31(70.5%)
109-140	51(29.7%)	29(28.2%)	12(48.0%)	10(22.7%)
>140	31(18.0%)	23(22.3%)	5(20.0%)	3(6.8%)

PON1-activity (U/L) mean±SD			
	67.45□8.9		48.12□25.6
Ar-activity (U/L) mean±SD			
	66.34□5.3		54.18□12.8
Note: The percentage is not equal to 100 due to the rounding effect			

The highest frequency of having more than 3 risk factors is observed in patients in the UA group, followed by those with NSTEMI and STEMI (Figure 3).

When examining the data from the patients included in Table 11, the distribution of risk factors can be observed, namely:

- with one RF are 15 (8.7%);
- with two RFs are 47 (27.3%);
- with three and more are 106 (61.6%).
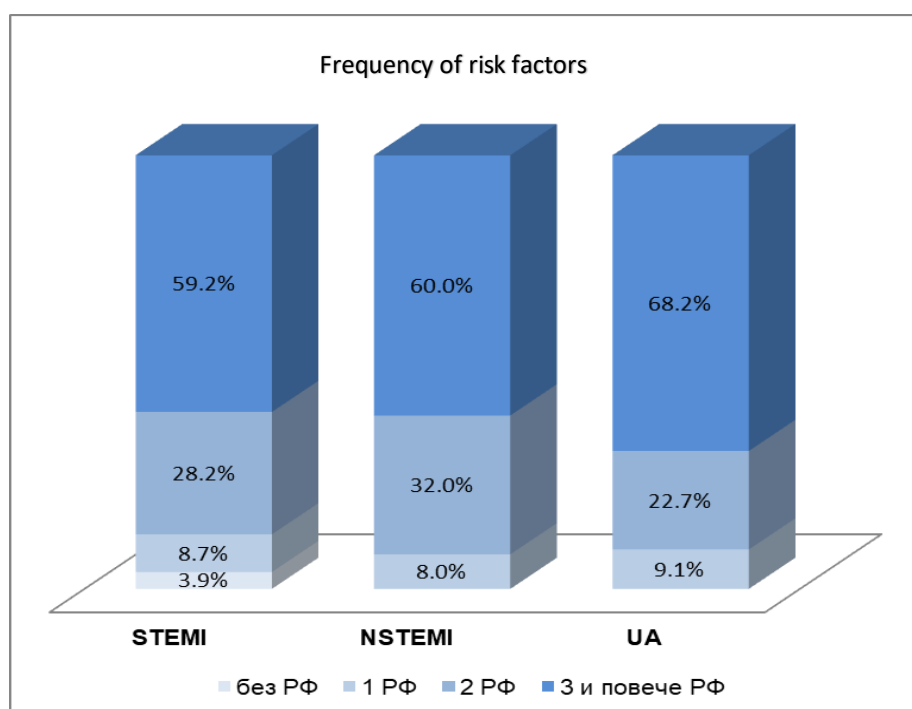
Figure 3: Distribution of RF in patients with acute coronary syndrome

A significantly larger number of patients with known prior stable angina is observed in the group with non-ST-elevation acute coronary syndrome (NSTE-ACS) (68.1%), unlike the group with STEMI (37.9%, p<0. 0001).

Determining the contribution of the time factor from the onset of clinical symptoms to the overall survival of patients.

The highest percentage of patients with all three diagnoses are seeking medical assistance after 24 hours as of the onset of chest pain (n=96, 55.8%). A relatively small number of patients with STEMI (32, 31.1%) sought medical assistance within 6 hours, and 45 (46.9%) only after 24 hours.

The timely or early seeking of medical assistance is not related to the gender and age of the patients.

No association was found between the time of the onset of the clinical symptoms and the overall survival in the hospital on the 6th, 12th, 24th and 108th month.

Relationship with diagnosis

In Table 4A are represented the mean values of the indicators of the lipid profile in the overall group, as well as in the separate groups based on diagnosis.

The highest value of the total cholesterol (TC) and β-cholesterol (ЛДЛ) is in the group of STEMI, and there is a tendency for significant difference from UAP (p=0.072 and p=0.047, respectively). No significant differences were found in the other indicators between the other diagnostic groups.

Relationship with gender

When analyzing the lipid profile indicators based on gender (Table 4B), no significant difference in the value of TC by gender was found (p=0.203). There is no difference in the levels of β-cholesterol (LDL) (p=0. 217), as well as in TG (0. 527) too.

Relationship with age

When dividing the patients into subgroups based on age, we observed a descending gradient in total cholesterol and LDL-C among men. (p=0.049, p=0.069, respectively, ANOVA test). For HDL-C and TG we identified statistically significant differences in the levels among the diverse groups (p=0.013, p=0.009, respectively, ANOVA test). Significantly lowest values of HDL-C have the young men under 49 years (p=0.003, p=0.076, LSD post hoc), and the elderly men over 70 years have significantly highest values of TG (p=0.004, p=0.010, LSD post hoc) compared to the other age groups. (Figure 4)

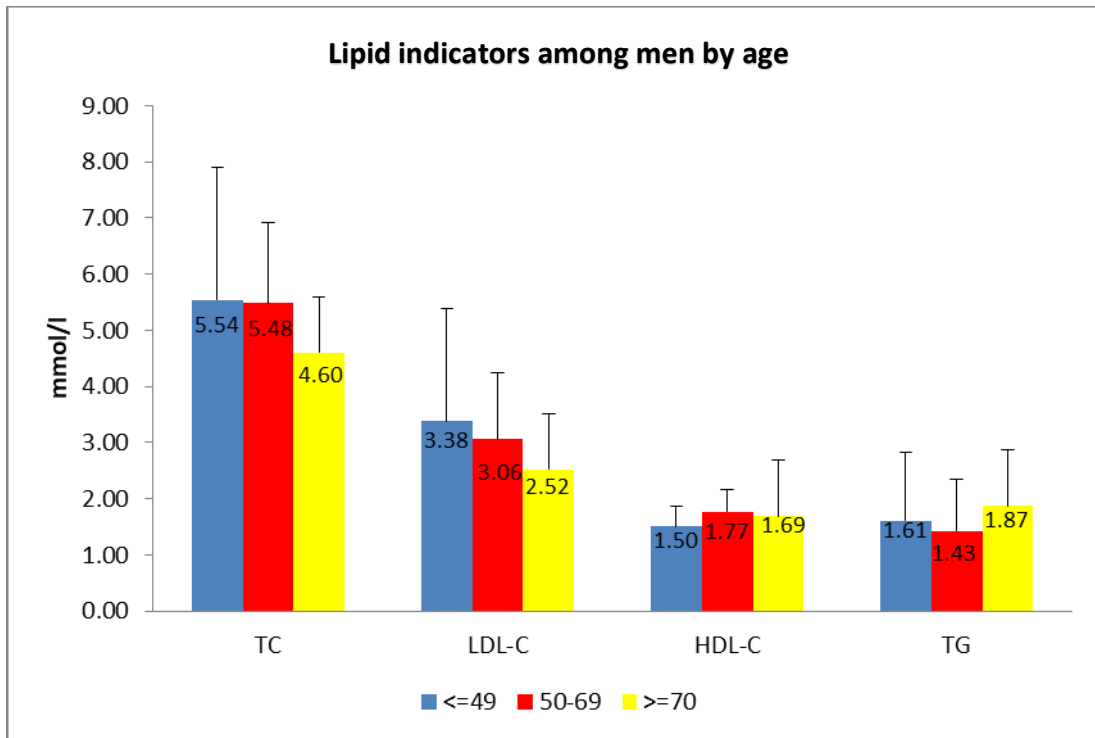No differences in the lipid indicators values have been identified among the age groups of women.

Figure 4. Lipid profile indicators among men in the diverse age groups.

Relationship with BMI

The value of TC and LDL in our group does not correlate with BMI, using the Spearman's rank correlation coefficient. We identified slight positive correlation between the elevated TG levels and the increased BMI (Spearman's coefficient Rho=0.294, p<0. 0001) (Figure 5A) and weak negative correlation between HDL and BMI (Spearman Rho= -. 180, p=0.019) (Figure 5B).
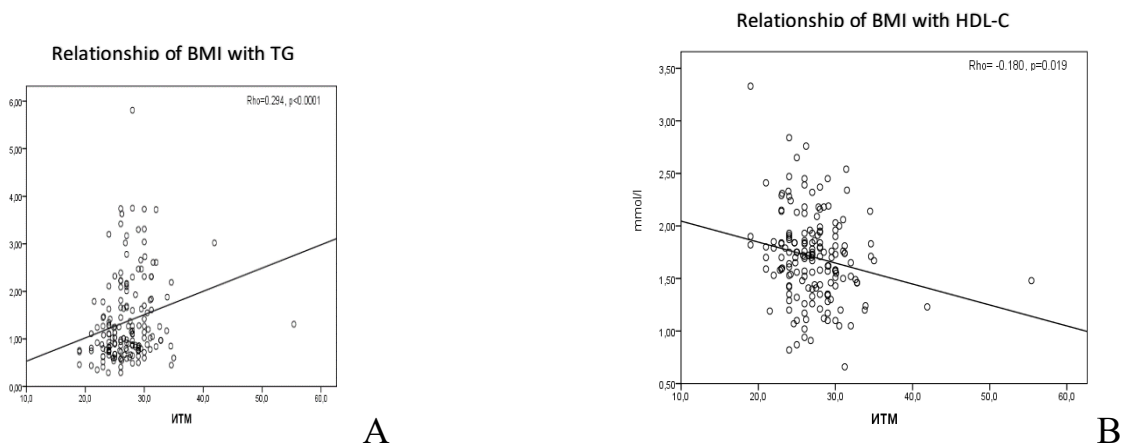

A

B

Figure 5: Correlation between BMI and the levels of TG (A)and high-density cholesterol (HDL-C) (B).

When examining the correlation relationships between BMI and lipid profile indicators in both genders, it was found that the statistical significance of the correlations between BMI with TG and HDL-H is kept among women (Rho=0.370, p=0.004; Rho= -0.268, p=0.041, respectively), while among men only the correlation of BMI with TG (Rho=0.276, p=0.003) remains significant.

Correlation with the presence of diabetes mellitus

Regarding diabetes mellitus, we found that among individuals with diabetes, there is a tendency for lower values of TC (p=0.100), significantly lower values of low-density lipoprotein cholesterol (p=0.010), and significantly higher values of triglycerides compared to non-diabetic individuals (p=0.043). The high-density cholesterol was higher among individuals without diabetes without presence of statistical significance (p=0.539). (Figure 6)
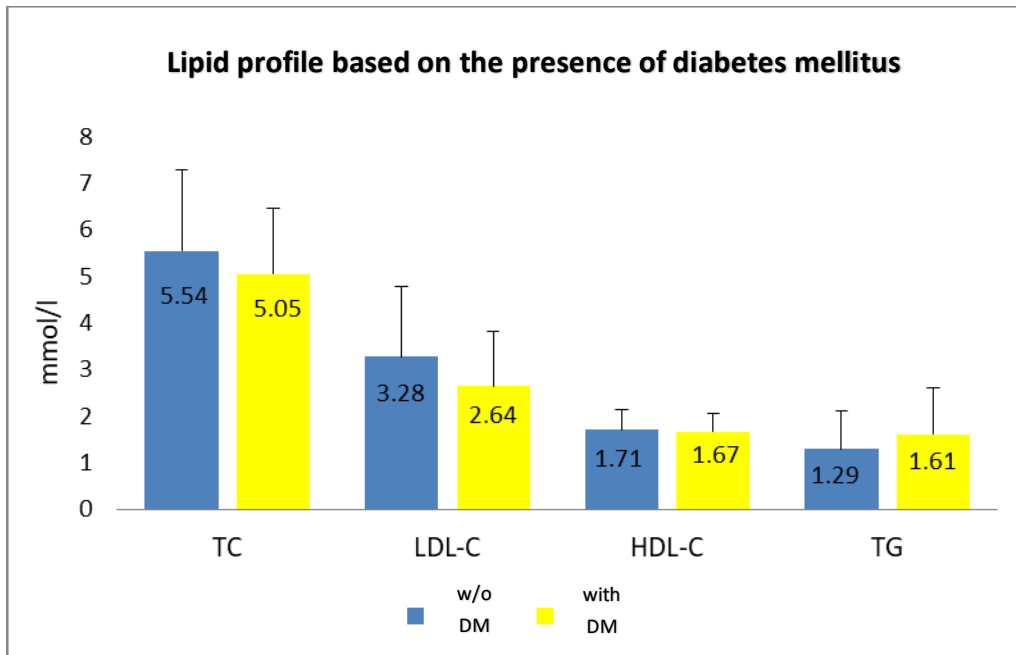
Figure 6: Lipid profile indicators values with/without diabetes mellitus (DM).

Correlation relationship with smoking

Among smokers, there is a higher level of TC and LDL-C, although statistical significance has not been reached. Regarding TG, a statistically significant difference was found among the three groups (p=0.002, ANOVA test), ex-smokers having significantly higher values compared to the other groups (p<0.0001, p=0.003, LSD Post Hoc) (Figure 14).
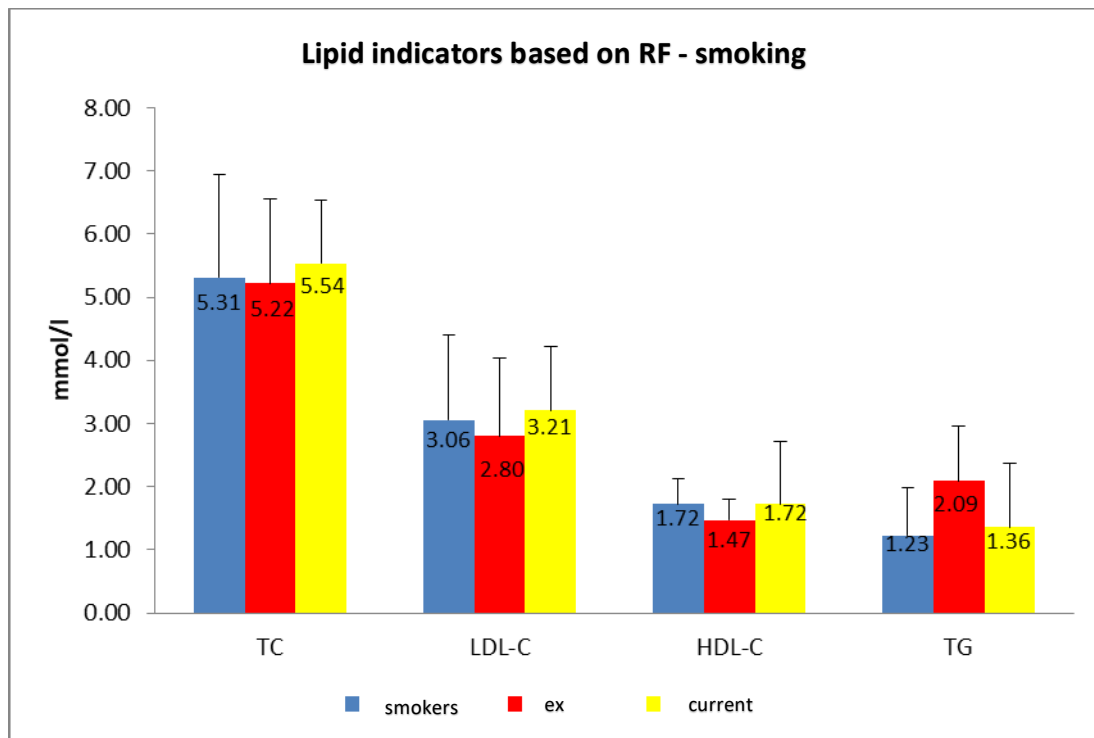
Figure 7. Correlation between lipid levels and smoking in the entire patient group.

When examining the same correlations in both genders, no statistically significant differences have been identified, except for TG among men (P<0.0001, ANOVA test), ex-smokers having significantly higher values compared to the other groups, non-smokers and current smokers, respectively (p<0.0001, p=0.001, LSD Post Hoc).

Correlation with arterial hypertension

No correlation has been identified between the presence of arterial hypertension and serum values of TC (p=0.769), LDL-C (p=0.548), HDL-C (p=0.673) and TG (p=0.695). Comparable results were observed when disaggregating by gender.

Correlation with family history

The presence of family history of early CAD was not associated with the examined biochemical lipid profile markers. When examining these correlations among both genders it was identified only that TG are significantly higher in

women with family history (1.79±0.91 mmol/l) compared to those without family history (1.33±0.68 mmol/l, p=0.045).

Correlation with diagnosis

The results in Table 4B show that there is a tendency of difference in the mean value of the glomerular filtration (GFR) in the different diagnoses (p=0.089), the values being significantly higher in NSTEMI compared to STEMI (p=0.036), and insignificantly higher compared to UAP (p=0.101).

When separating the patients into subgroups in accordance with the limit value for GFR (<60 ml/min), a statistically significant difference was identified between patients with different diagnosis. The patients with STEMI diagnosis with GFR<60ml/min are significantly more than those in the group with UAP (p=0. 006), and the patients with NSTEMI rarely have values of GFR<60 ml/min from UAP (p=0. 041).

Correlation with gender

The analysis of the examined parameters in both genders showed that the glomerular filtration rate (GFR) in men was significantly higher compared to women (p=0.001) (Table 4) and without significant difference in the values of the creatinine (p=0.116) (Table 4A).

Correlation with age

Our results confirm that with advancing age GFR decreases significantly (Rho= -0.514, p<00001) (Figure 8), and the serum creatinine increases (Rho= 0.340, p<00001). When analyzing these correlations in both genders, the significance was maintained.
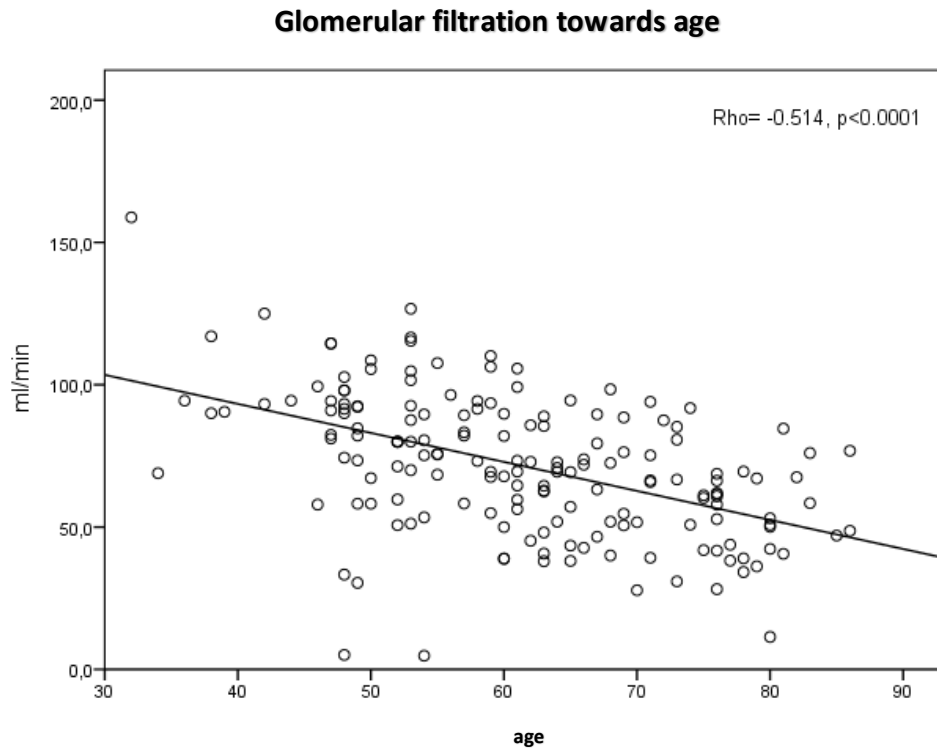
Figure 8. Correlation of GFR with patients' age.

Correlation with presence of diabetes mellitus

When analyzing the GFR and the serum creatinine in patients with and without diabetes mellitus, it was discovered that the patients with diabetes mellitus have significantly lower GFR (p=0.039), while the serum creatinine had equivalent values (p=0.979). The breakdown by gender showed that the values of the examined indicators do not differ in women with/without DM, while only GFR in men with DM showed significantly lower values compared to those in men without diabetes (p=0.036) (Figure 9).
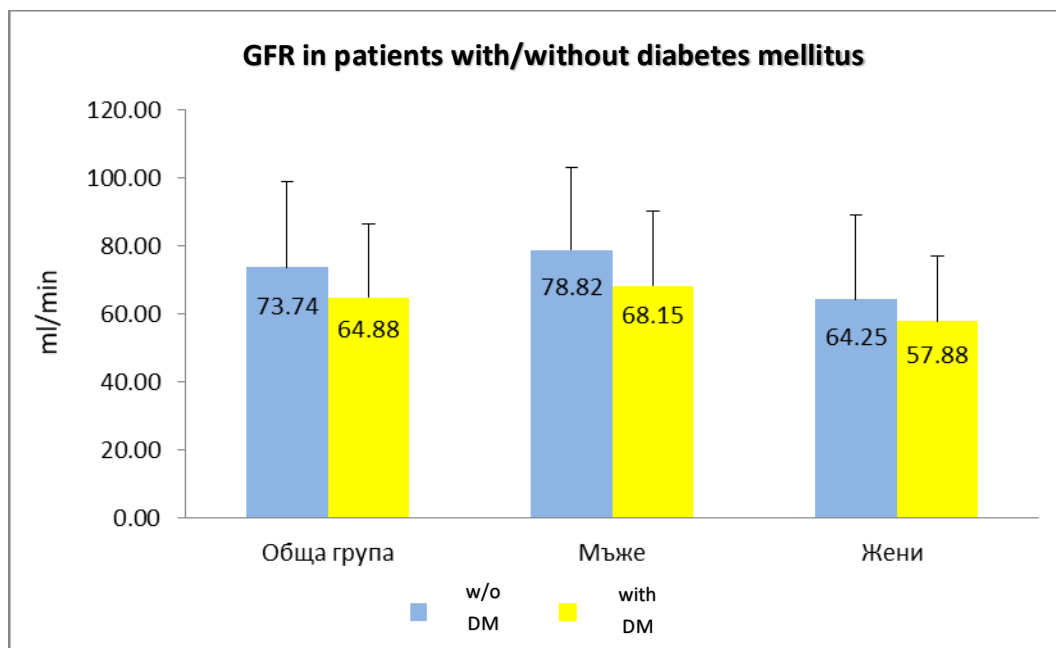
Figure 9. Values of GFR in the general group and by genders depending on the presence of diabetes mellitus (DM).

Correlation with smoking

The result was interesting and showed significantly higher values of GFR in current smokers (84.29±24.38 ml/min) compared to ex-smokers (75.99±18.24 ml/min, p=0.021, LSD Post Hoc) and non-smokers (61.57±21.14 ml/min, p<0.0001, LSD Post Hoc). Analogical results have been received in the different age groups (except the oldest age group), as well as when grouping by gender.

Correlation with arterial hypertension

We discovered significantly lower values of GFR (p<0.0001) and a tendency for higher values of serum creatinine (p=0.166) in patients with arterial hypertension. Regarding GFR by gender, the differences remain statistically significant (p=0.013 in women; p=0.001 in men). (Figure 10).
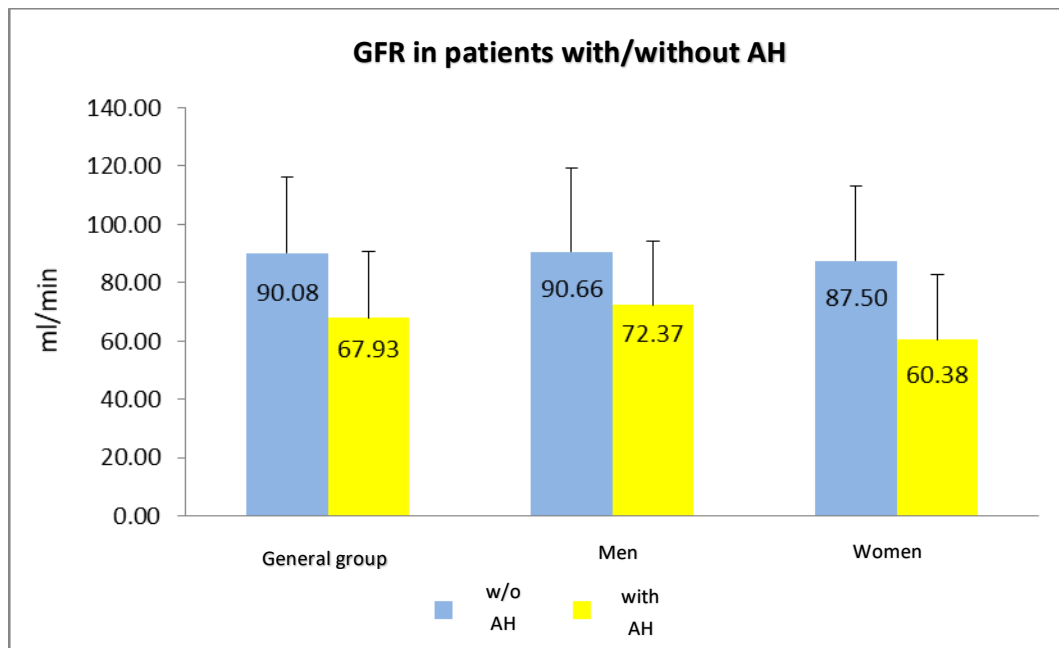
Figure 10. Values of GFR in the general group and by genders depending on the presence of arterial hypertension (AH).

4.2.3. Analysis of the correlation of the cardiac enzymes – CPK, CPK-MB, Tn with demographic and risk factors;

Correlation with diagnosis

As shown in Table 5, the highest values of the examined indicators are observed in patients with STEMI, and a statistical significance was identified for CPK and CPK-MB fraction ($p<0.0001$ for both indicators, ANOVA test).

Correlation with risk factors

No correlations have been identified between cardiac markers with BMI, the presence of DM and AH. Interesting result has been obtained for the levels of troponin in current/ex-smokers and non-smokers ($p=0.008$): ex-smokers had significantly higher levels of troponin than the non-smokers ($p=0.003$), especially towards the current smokers ($p=0.004$).

In the group of STEMI (30/100, 39.0%) and NSTEMI (11/25. 44%) have EF between 40-49% compared on UAP, where only 11.4% (n=5) have such value (p=0.027). Contrary, a much larger proportion of patients with hypertension have a reduced ejection fraction > 50% (35/44, 79.5%) compared with the other two groups (Figure 11).
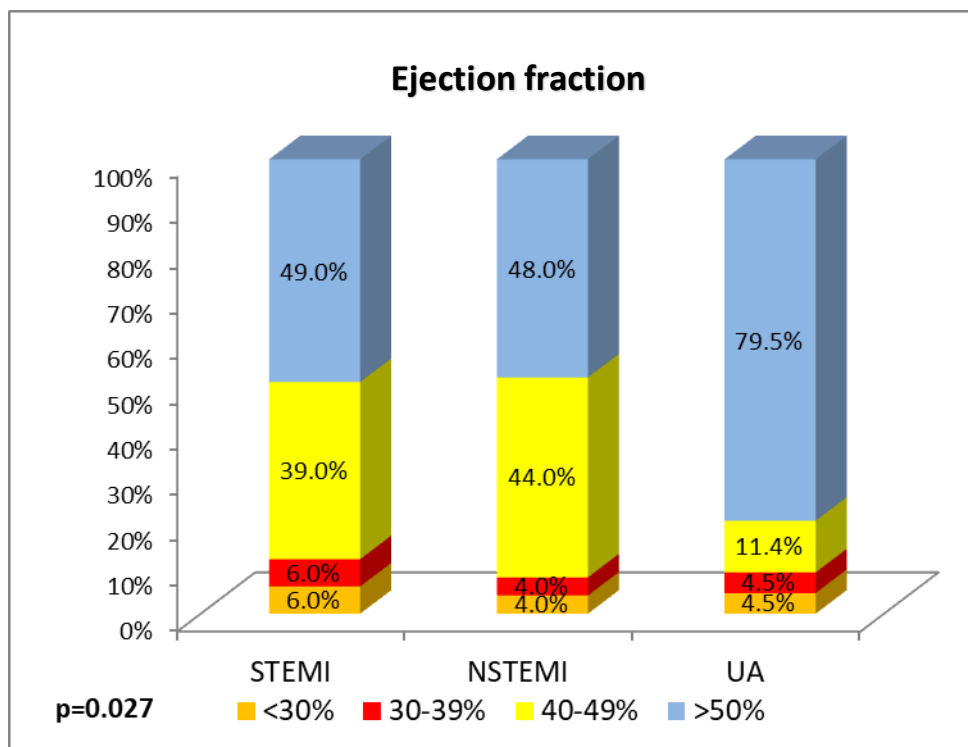
Figure 11. Ejection fraction in patients with ACS.

After applying the Tukey's multiple comparison test, we found that patients with EF>50% have average age of 58.7±10.9 years, and those with EF within the range 49-40% of 66.4± 12.1 years (p=0.001, Tukey's test) (Figure 12).
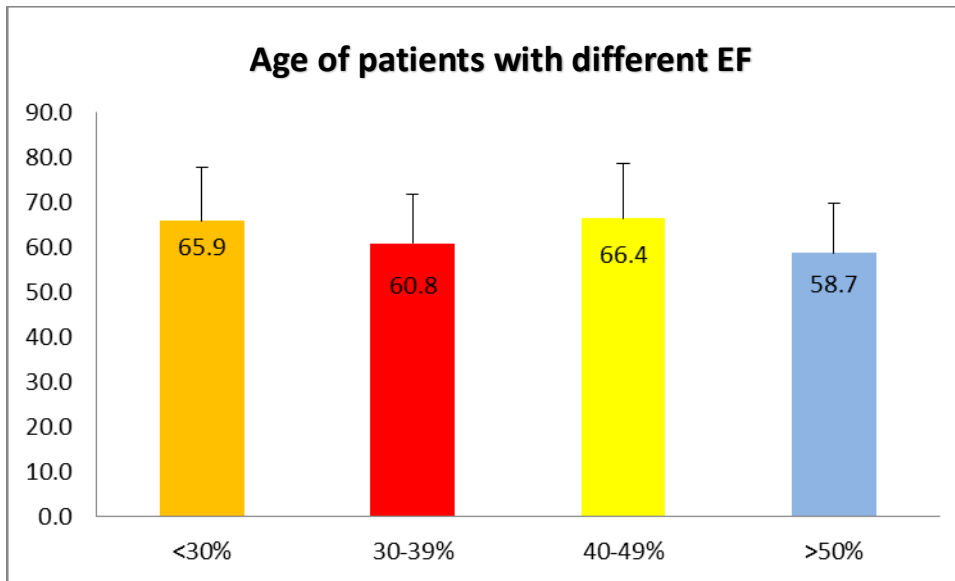
Figure 12. Average age of the patients with different ejection fraction (ANOVA test, p=0.001).

Analyzing the correlation between the number of affected vessels and the ejection fraction in the overall group, we found that patients without angiographic data on stenosis, as well as those with single-vessel coronary disease, more frequently have an ejection fraction upon hospitalization above 50% (90% and 62.0%, respectively) compared to patients with double- and three-vessel coronary disease (57.8% and 39.6%, respectively, p=0.013). Patients with ACS who have occlusion of the left anterior descending artery (LAD) more often (54.2%), have ejection fraction at admission lower than 49% compared to those who do not have occlusion of this artery (28.1%) (p=0.002).

A significantly lower percentage of patients with EF <30% survived after one year (44.4%) compared to patients with higher ejection fractions (p=0.017) (Figure 13A). Up to the fifth year and to the ninth year, the survival depended again on the ejection fraction at hospitalization (Figure 13 B and C).

Survival at 12th month based on EF

A

Survival at 60th month based on EF

B

Survival at 108th month based on EF
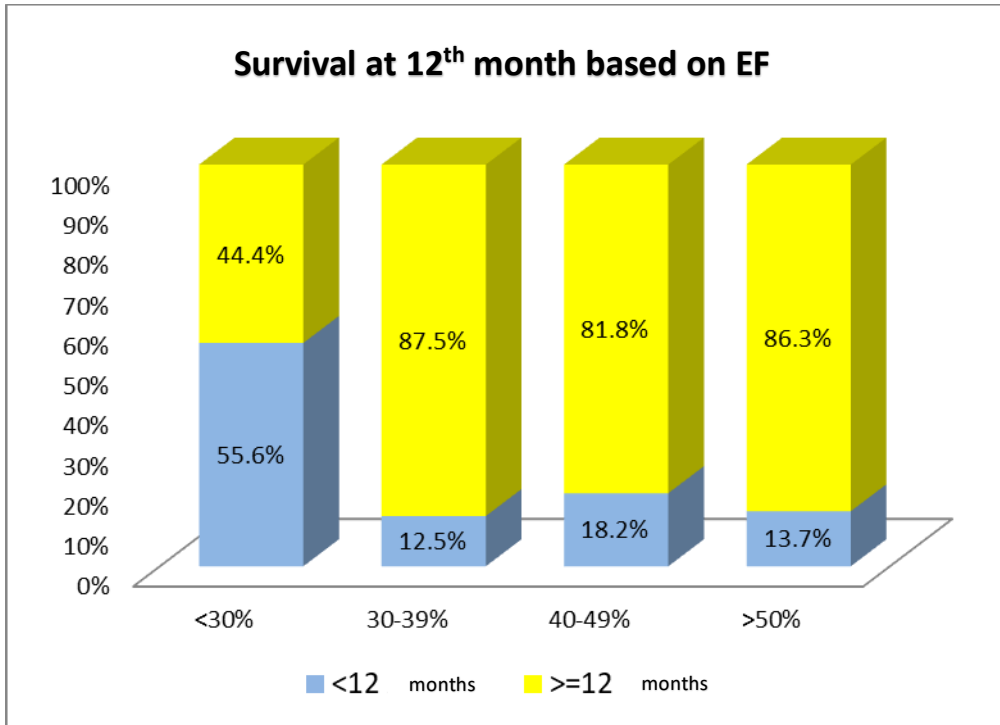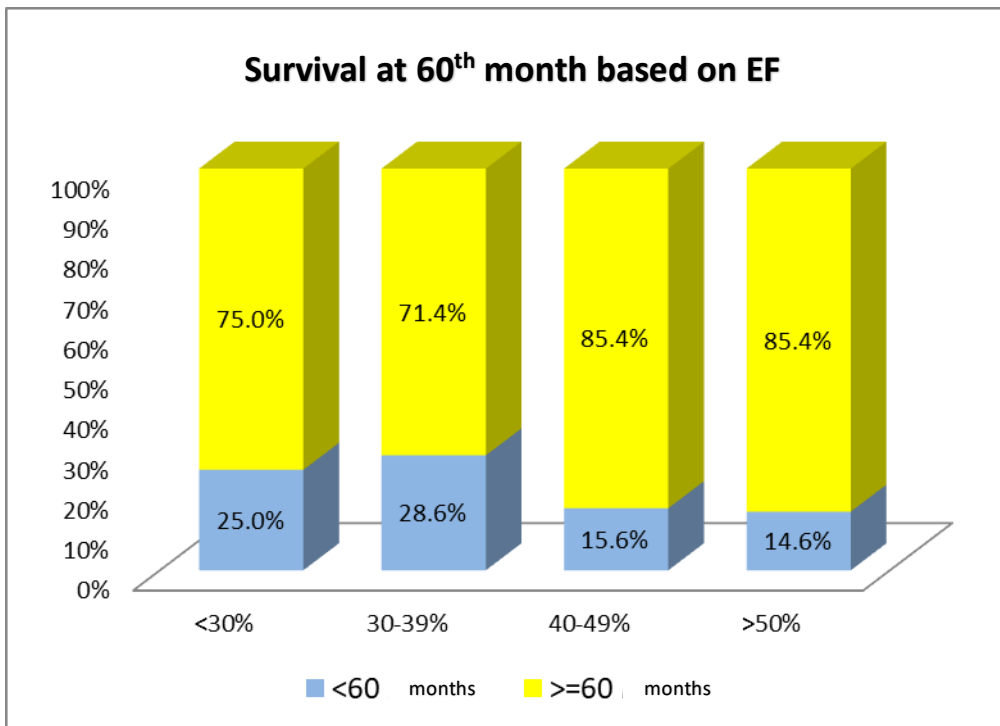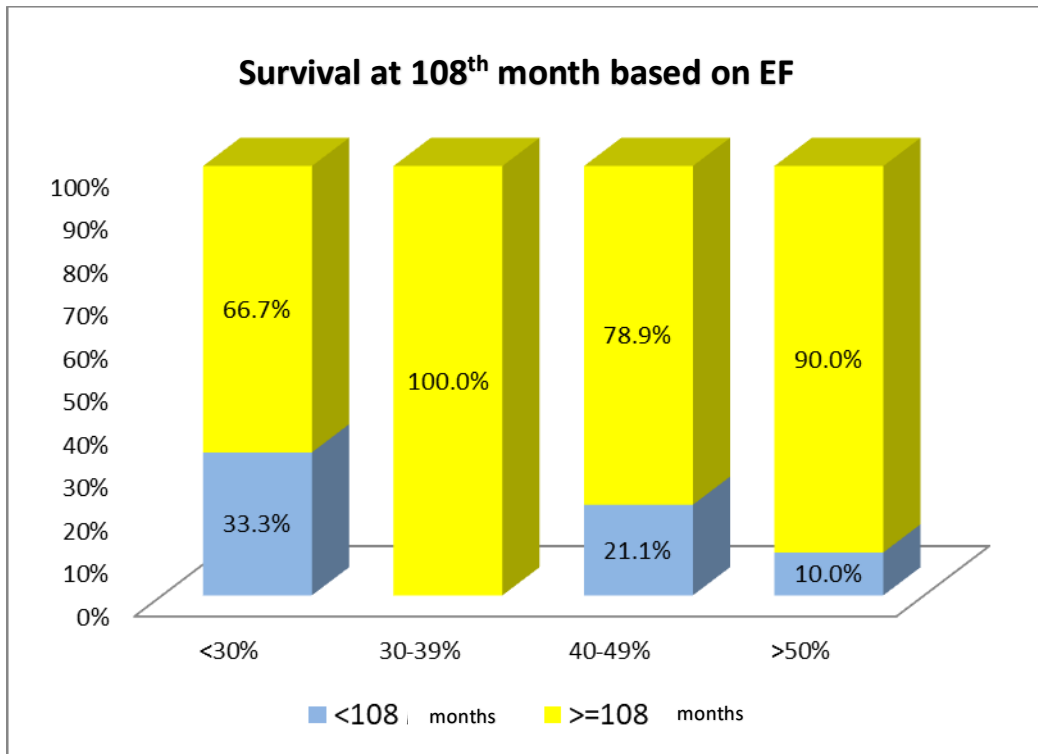
C

Figure 13: Patient survival rates based on ejection fraction (EF) at 12th month (A), 60th month (B) and 108th month (C).

4.4. Analysis of the morphological changes discovered during invasive examination with the known risk factors.

The distribution of patients with single-vessel, double-vessel, and triple-vessel coronary artery disease based on coronary angiography is respectively 51, 46 and 48 patients (Figure 21).
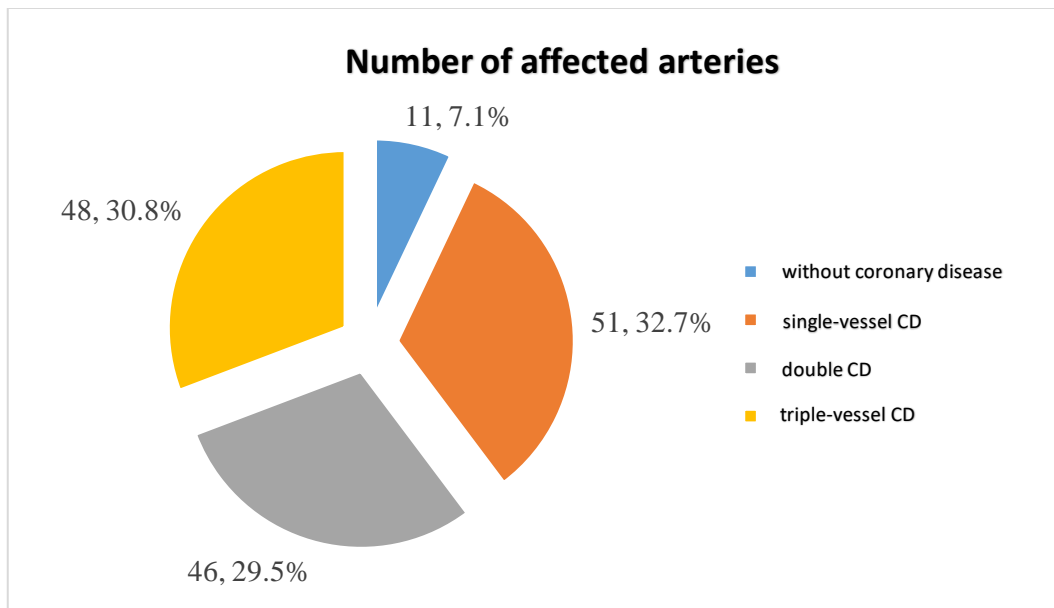
Figure 14. Number of the patients with single-, double-, and triple-vessel coronary disease (CD) and without CD.

A statistically significant difference in the frequency of the number of affected vessels was observed among the different diagnoses ($p<0.0001$) (Figure 14). Triple-vessel coronary disease occupies the largest share in NSTEMI (52%), while in UAP is 30%, and in STEMI 25.7%. Logically patients with STEMI have most often single-vessel CD (39.6%).
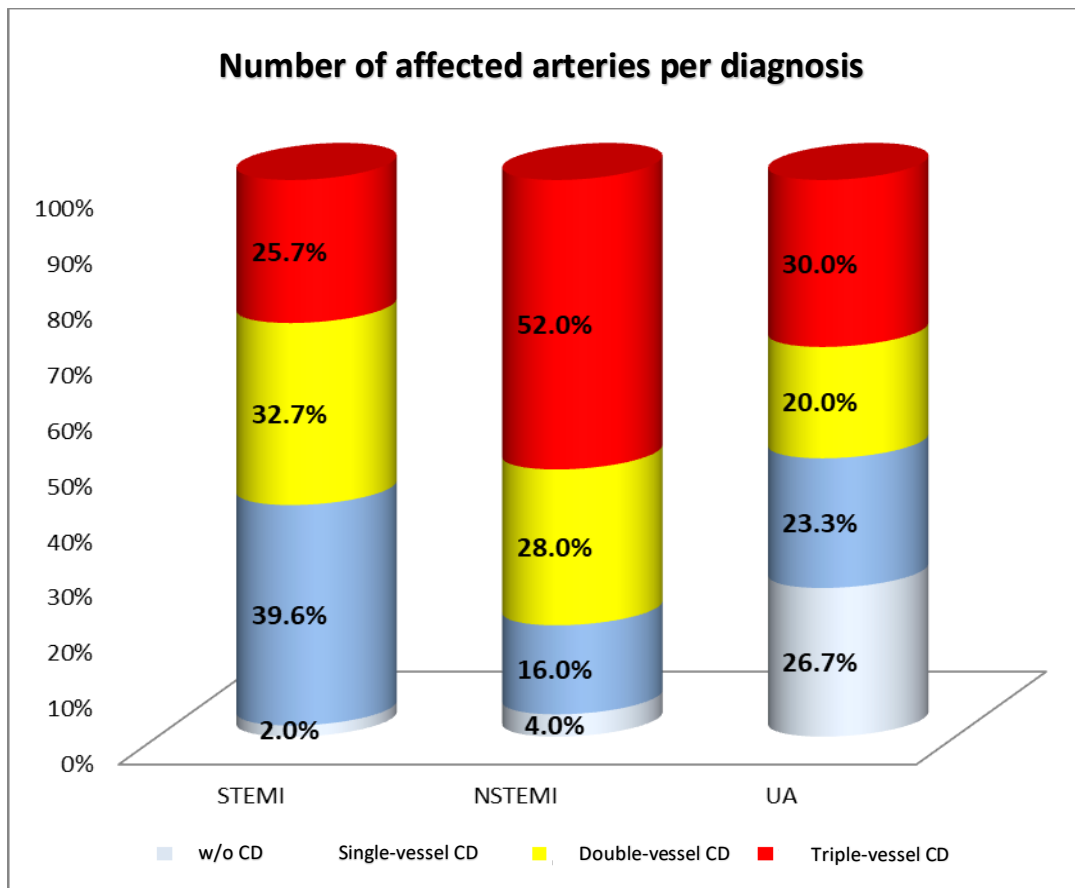
Figure 15. Number of patients with single-, double-, triple-vessel disease (CD) and without CD based on diagnosis.

The age of the patients with one affected coronary vessel is significantly lower than the one of the patients with two (p=0.016, LSD Post Hoc analysis) and three affected vessels (p<0.0001, LSD Post Hoc analysis) (Figure 16).
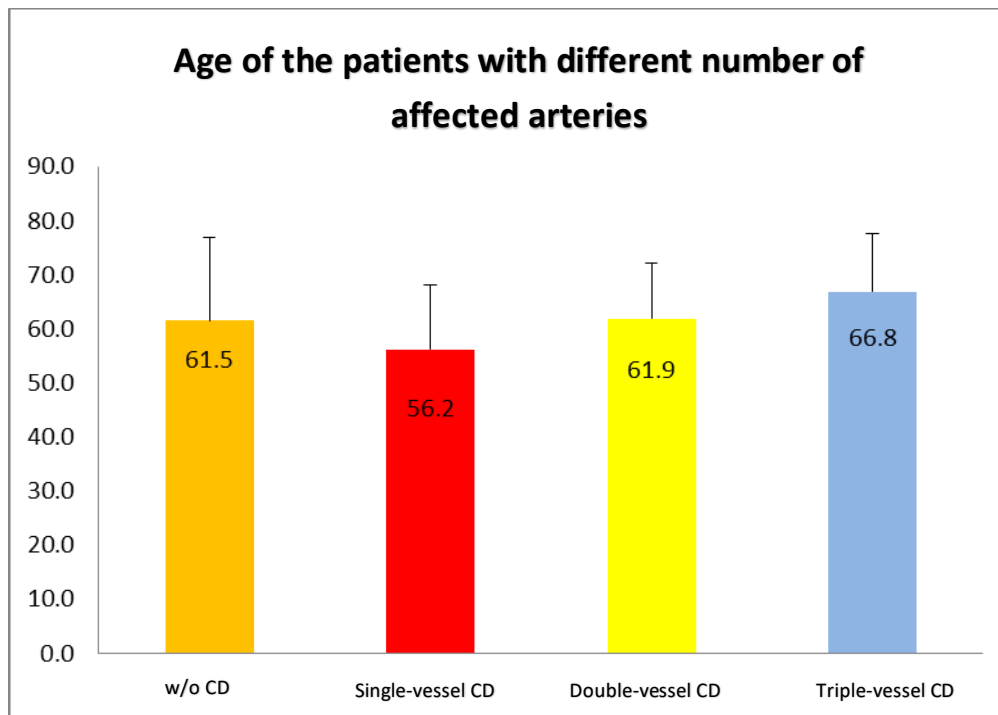
Figure 16. Age of the patients with different number of affected arteries (p<0.0001, ANOVA test).

Patients without medical history for AH more often have single-vessel CD (58.3%), while those with AH have more often double-vessel (32.6%) and triple-vessel (32.6%) CD (p=0.024).

In terms of glomerular filtration rate values (GFR) there is a tendency for triple-vessel involvement in patients with GFR<60 ml/min (41.5%), in comparison with the patients with GFR over 60 oml/min (24.8%) (p=0.098).

In the follow-up of patient survival at 12 months, 5 years, and 9 years, we observed that the 12-month survival is not influenced by the number of the affected by atherosclerosis coronary vessels (p=0.349), while at fifth year the mortality in case of triple-vessel involvement is significantly higher (33.3%), than in patients from the other groups (9.1% in single-vessel CD, 15.4% in double-vessel CD, and 14.3% in patients without CD (p=0.041). This significance is lost at the ninth year of the follow-up (p=0. 389).

There is a tendency for higher in-hospital mortality in patients with triple-vessel involvement and high GRACE (p= 0. 003). A statistically significant relation has been identified between the presence of acute left ventricular heart

failure and death during hospitalization (p=0. 017), regardless of the culprit vessel (LM, LAD, LCx, RCA), as reason for ACS.

The in-hospital mortality was seven. 6%. Cardiac arrest has been registered at 13 (7.6%).

The calculated GRACE-score for in-hospital mortality by diagnosis is presented in Table 5. A significantly higher number of patients diagnosed with STEMI have an elevated risk of mortality (22.3%) i.e., GRACE>140 p., in contrast to those with UAP (6.8%) and NSTEMI (20.0%) (p=0.013). The calculated GRACE by gender is presented in Table 5.

Table 5. Distribution of the GRACE marker by gender.

GRACE	Women	Men	p-value
Minimal risk	24(40.7%)	66(58.4%)	
Moderate risk	17(28.8%)	34(30.1%)	0.006
Considerable risk	18(30.5%)	13(11.5%)	

This association between gender and the GRACE score is maintained at the 6th month, with a significantly higher proportion of women (47.5%) being at considerable risk of death compared to men (29.2%) ($\chi2(2)$=6.004; p=0.048). We have associated the individual risk score and we found a significant association between the calculated GRACE-score and the favorable treatment outcome during hospitalization (p=0.001). The survived patients (25.8%) during in-hospital treatment had GRACE<89 p., and among deceased there were no estimated with minimal risk (0. 0%).

4.6. Defining the serum paraoxonase and arylesterase activity of PON1, distribution and frequency rate of the examined polymorphic variant L55M PON1 in patients with ACS and healthy control subjects. Analysis of the correlation between the abovementioned with the biochemical indicators and the survival rate.

4.6.1. Studies of the serum paraoxonase and arylesterase activity of PON1 in patients with ACS and healthy control subjects;

In the studies of the enzyme activities of PON1 were included 42 patients, of which: 36 had acute myocardial infarction with ST-elevation (STEMI) and 6 patients had unstable angina (UA). The mean values of paraoxonase and arylesterase activity in patients and control subjects had statistically significant differences, with significantly lower values observed in both STEMI (p=0.0008 for PON- and for ArEs activity) and UA (p=0.0005 for PON activity и p=0.002 for ArEs activity respectively) patients (Table 6) (Figure 17A and 17 B and Figure 18A and 18B).

Table 6. Mean values of the serum paraoxonase and arylesterase activity of PON1 and their normalized values of HDL cholesterol in control group and the groups of patients with UAP and STEMI.

	Patients		Control groups	p-value	
Indicators	UAP	STEMI		UAP	STEMI
PON-activity(U/L)	48.12± 25.6	67.45± 8.9	128.79± 15.1	0.008	0.0005
ArEs-PON1(U/L)	54.18 ± 12.8	66.34± 5.3	88.38± 3.0	0.008	0.002
PON1/HDL-C	24.30±28.81	36.84±28.99	89.45±64.40	0.003	<0001
ArEs/HDL-C	37.21±11.29	36.91±11.38	60.83±24.9	0.037	0.0008
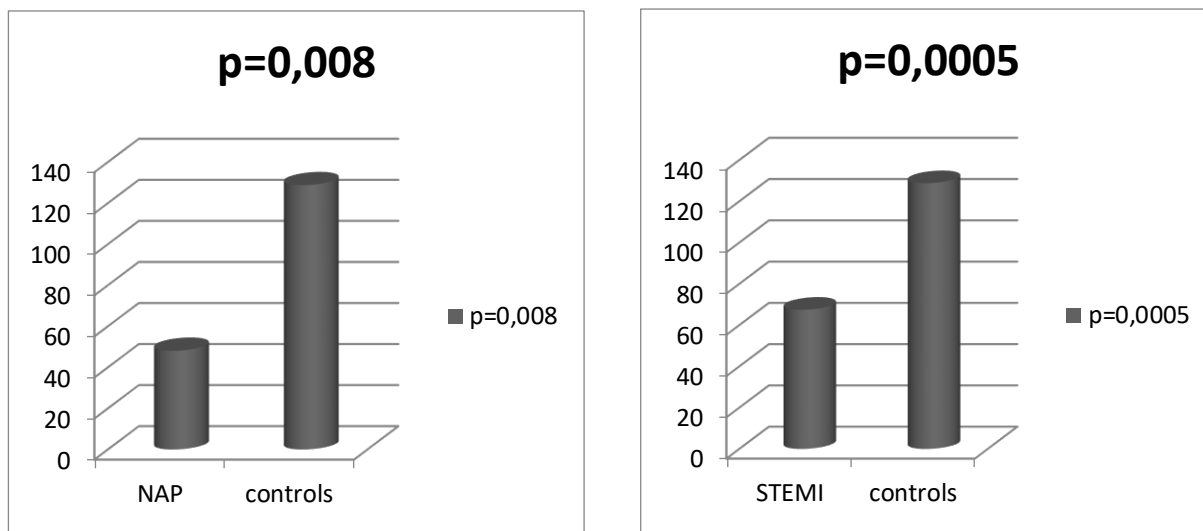
Figure 17. Serum paraoxonase activities of PON1 in UA (A) and NSTEMI (B) patients and healthy controls.
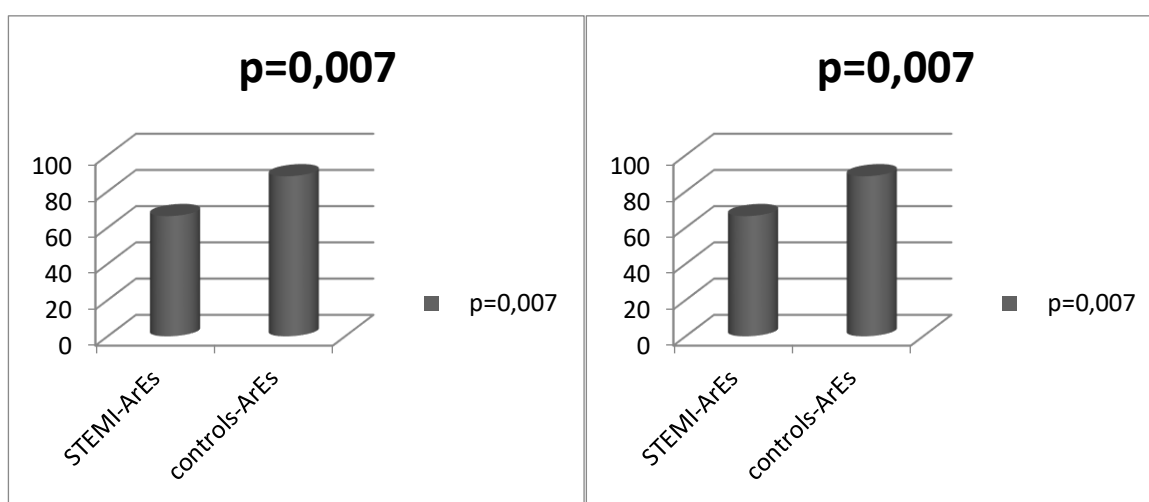
Figure 18. Serum arylesterase activities of PON1 in UA (A) and STEMI (B) patients and healthy control subjects.

Analogically, statistically significant differences have been observed when comparing the normalized values of HDL cholesterol of the paraoxonase and arylesterase activity (Table 6).

When comparing the paraoxonase activity of the PON1 enzyme with the extent of coronary artery disease (single-vessel, double-vessel, triple-vessel, and multi-vessel), we observed a tendency for lower values in cases of triple-vessel and multi-vessel coronary artery disease (p=0.07). The normalized values of the paraoxonase activity of PON1 towards the levels of HDL-C in triple-vessel and

multi-vessel coronary disease are lower than the single-vessel coronary disease without statistical significance (p=0.07).

When comparing the paraoxonase activity between individuals with arterial hypertension (AH) and individuals without any historical or instrumental data of AH, we observed statistically significantly lower values in patients with existing arterial hypertension (p=0.02). When comparing the arylesterase activity in individuals with AH and without any anamnestic or instrumental data for AH, we obtained statistically significantly lower values in patients with existing arterial hypertension (p=0.03). The normalized values of the PON-activity of PON1 in terms of the levels of HDL-C (PON/HDL-C) in patients with anamnestic or instrumental data for AH are significantly lower than those without AH (p=0.03).

The average paraoxonase activity of PON1 in individuals who have experienced a cardiovascular incident is significantly lower compared to those without a previous cardiovascular incident (p=0.018). We obtained significantly higher values of total serum cholesterol in individuals with acute myocardial infarction and unstable angina compared with the control group (p<0.001, p<0.0069). The patients with acute myocardial infarction without/with ST-elevation and UA had higher values of the body mass index (BMI) in comparison with the control group (p=0.0027, p=0.046).

There was a tendency for a decrease in serum activity of PON in diabetes mellitus compared to the individuals without diabetes (p=0.05), while the arylesterase activity did not show significant variations (p=0.20).

When analyzing the group of the control subjects, we identified a correlative dependency between the serum paraoxonase activity of PON1 and alfa-cholesterol in control subjects (p=0.895) (Figure 19A).

When analyzing the correlation dependencies between the levels of serum PON activity of PON1 of the patients with HDL-cholesterol, a tendency for weak positive correlation has been identified (R=0.273, p=0.080) (Figure 19 B). There was a weak negative correlation, but not statistically significant, between serum

levels of paraoxonase activity of PON1 and β-cholesterol (LDL-C) in patients (R=-0.199, p=0.213) (Figure 19 C).
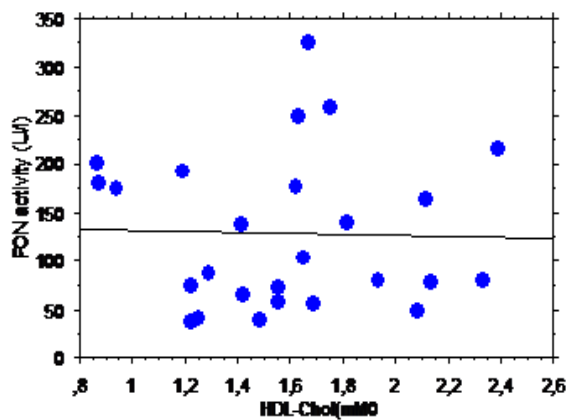
Figure 19A
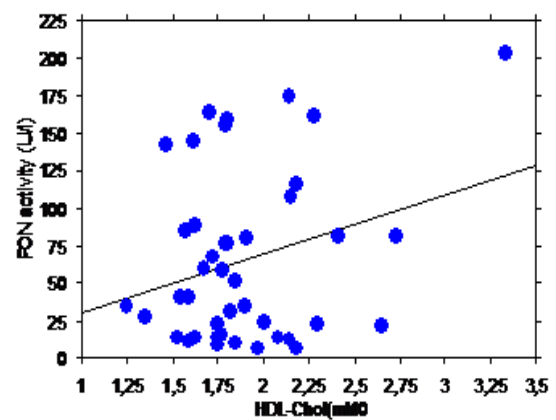
Figure 19B
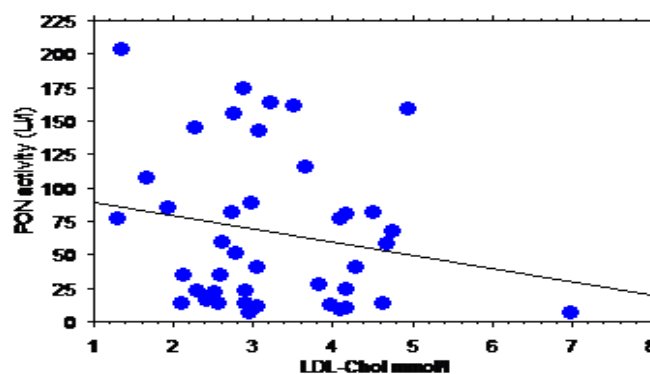
Figure 19C

Figure 19. Correlations of the serum paraoxonase activity of PON1 with HDL-cholesterol in control subjects (A), in patients (B) and with LDL-cholesterol in patients (C).

4.6.2. Determining the distribution and frequency of the polymorphic variant L55M PON1 in patients with ACS and healthy control subjects.

The results from the genotyping for PON1 L55M (163T>A, rs 854560) SNP showed statistically significant difference (p=0.023) between the control subjects and the entire group of patients with acute coronary syndrome (Table 7). This distribution of the genotype defines more than two times higher risk of developing acute coronary syndrome, when the individual is carrier of the genotypes with M allele variant of PON1 L55M (Table 7). The statistical

significance has been maintained even after correction by gender and age (Table 7).

Table 7: Genotype and allele distribution of L55M in PON1 in patients with acute coronary syndrome and control subjects.

L55M SNPin*PON1*	Patients number (N) n=77	Frequency	Control subjects number (N) n=122	Frequency	OR (95% CI). p-value	OR (95% CI). p-value (after correction by gender and age)
Genotype distribution						
LL	28 (36.4%)	0.364	69 (56.6%)	0.566	1.0 (referent)	
LM	39 (50.6%)	0.506	42 (34.4%)	0.344	2.288 (1.233; 4.248). **p=0.009**	2.390 (1.230; 4.643). **p=0.010**
MM	**10 (13.0%)**	**0.130**	**11**	**0.090**	2.240 (0.856; 5.865). **p=0.100**	**2.275** (0.973; 7.782). **p=0.056**
LM+MM	49	0.636	53	0.434	2.278 (1.268; 4.095). **p=0.006**	2.457 (1.306; 4.622). **p=0.005**
Allele distribution						
55L	104	0.627	180	0.738	1.0 (referent)	
55M	62	0.373	64	0.262	1.677 (1.098; 2.561). **p=0.017**	

When the genotype distribution was analyzed in the groups of patients with different diagnosis and was compared with the distribution in the control subjects, we identified that there is a significant difference only between the patients with STEMI and the control subjects (p=0.012) (Table 8), while there is no difference in the distribution of the genotype between the controls subjects and the patients with UA (p=0.433) and NSTEMI (p=0.819) (Table 8).

Table 8: Genotype and allele distribution of L55M in PON1 in patients with different diagnosis and control subjects.

L55M SNPin*PON1*	Patients n =77	Frequency	Control subjects n = 122	Frequency	OR (95% CI). p-value	OR (95% CI). p-value (after correction by gender and age)
Genotype distribution – patients with STEMI (n=53)						
LL	17	0.321	69	0.566	1.0 (referent)	
LM	29	0.547	42	0.344	**2.803 (1.376; 5.706). p=0.005**	**2.750 (1.304; 5.798). p=0.008**
MM	7	0.132	11	0.090	2.583 (0.872; 7.652). p=0.087	3.029 (0.950;9.658). p=0.061
LM+MM	36	0.679	53	0.434	**2.757 (1.398; 5.436) p=0.003**	**2.801 (1.372; 5.718) p=0.005**
Genotype distribution – patients with UA (n =10)						
LL	4	0.400	69	0.566	1.0 (referent)	
LM	4	0.400	42	0.344	1.643 (0.390;6.920) p=0.499	1.834 (0.419;8.029) p=0.421
MM	2	0.200	11	0.090	3.136 (0.512;19.22) p=0.216	4.967 (0.713;34.63) p=0.106
LM+MM	6	0.600	53	0.434	1.953 (0.542;7.273) p=0.318	2.295 (0.590; 8.927). p=0.231
Genotype distribution – patients with NSTEMI (n =14)						
LL	7	0.500	69	0.566	1.0 (referent)	
LM	6	0.429	42	0.344	1.408 (0.443; 4.474). p=0.562	1.597 (0.462; 5.527). p=0.460

MM	1	0.071	11	0.090	0.896 (0.100; 8.005). p=0.922	2.301 (0.214;24.80). p=0.492
LM+MM	7	0.500	53	0.434	1.302 (0.430; 3.939). p=0.640	1.670 (0.505;5.523). p=0.400

So, individuals with genotype with at least one M allele variant of PON1 L55M (163T>A, rs 854560) SNP appear to have more than 2.5 times higher risk of developing STEMI, than those that are homozygous for the wild type L allele (LL genotype) (Table 8).

4.6.3. Associations of PON1 L55M SNP with biochemical and clinical markers of patients with ACS;

The analysis of the relationship of the genotypes with the serum characteristics of the lipid profile and the serum markers of the renal functions (creatinine and glomerular fraction) showed that in patients with genotype with M allele variant (LM+MM), suffering from ACS without ST-elevation (NSTEACS, i.e. UA or NSTEMI), the serum levels of the total cholesterol (TC) and triglycerides (TAG) are significantly higher than in patients with NSTEACS with LL genotype (p=0.022 for TC и p=0.015 for TAG) (Figure 20, Table 9).
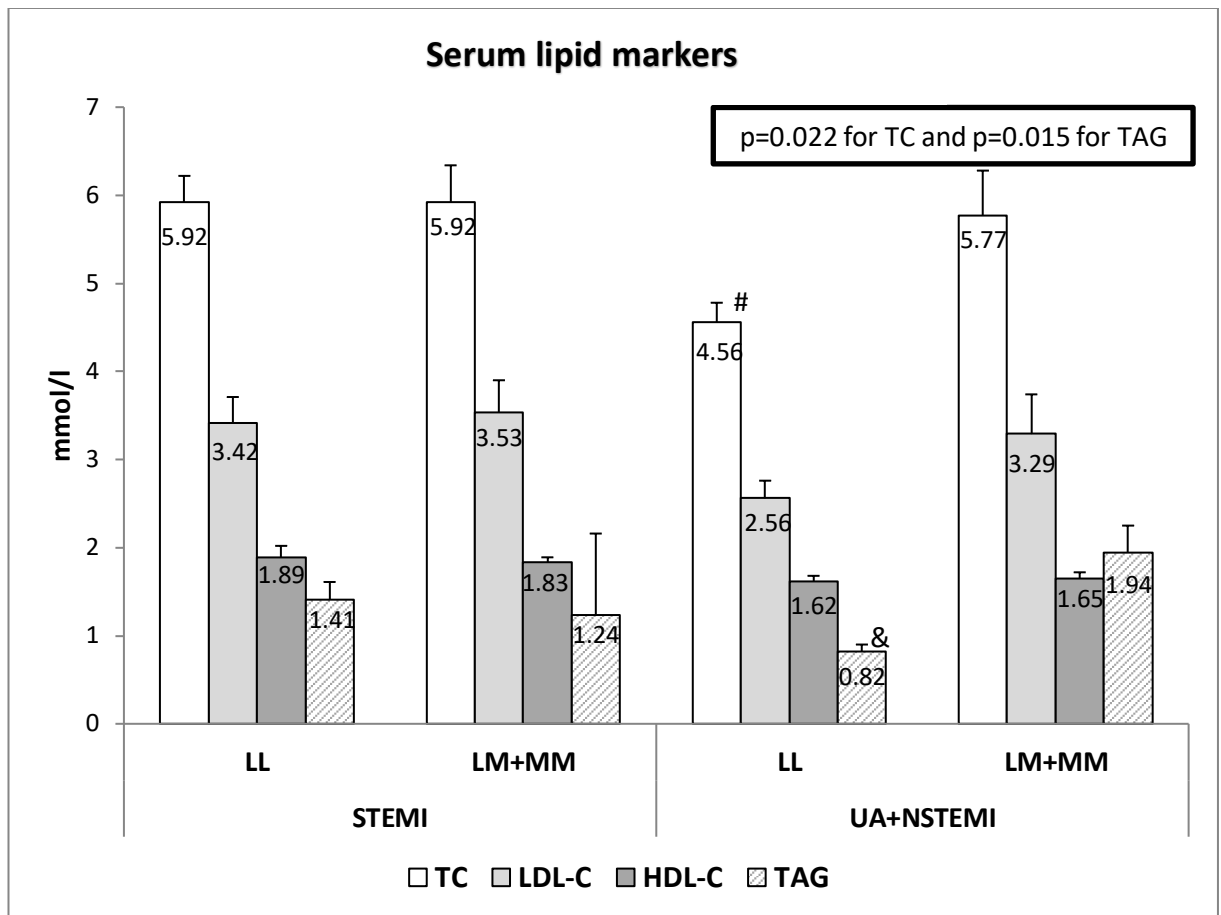
Figure 20: Serum lipid markers in the subtypes genotype with different allele frequencies in PON1.

When comparing the markers for renal function (Table 9), we found a tendency (p=0.072) without statistical significance for higher serum creatinine in patients with M allele genotype (LM or MM genotype), who are with NSTEACS, than in patients with the same diagnosis, but with LL genotype. When the patients were classified in groups based on the upper limit of the normal limits of 134 μmol/l creatinine, the tendency was preserved: patients with M allele genotypes (LM or MM genotypes), who are with NSTEACS, had more often higher creatinine (23.1%, 3/13) than the patients with the same diagnosis, but with LL genotype, who have normal values of creatinine (p=0.089). No difference in glomerular filtration rate (GFR) was observed among patients with different diagnoses and different genotypes (Table 9).

Table 9: Markers of the serum lipid profile and markers of renal function in patients with different PON1 L55M genotypes and diagnosis. The data are presented as mean SEM (standard error of the man value).

Indicators	STEMI			LL	NSTEACS (UA+NSTEMI)			LM+MM
	LL	LL_LM	p-value	p-value	LL	LL_LM	p- value	p-value
			(LL vs. LM+ MM)	(STEMI vs. UA+NS TEMI)			(LL vs. LM+ MM)	(STEMI vs. UA+NS TEMI)
TC (mmol/l) Mean±SEM	5.92±0.30	5.92±0.43	0.112	**0.005**	4.56±0.22	5.77±0.51	**0.022**	0.919
LDL-C (mmol/l) Mean±SEM	3.42±0.29	3.53±0.37	0.265	**0.042**	2.56±0.21	3.29±0.45	0.106	0.768
HDL-C (mmol/l) Mean±SEM	1.89±0.13	1.83±0.06	0.864	0.208	1.62±0.06	1.65±0.08	0.955	0.113
TG (mmol/l) Mean±SEM	1.41±0.20	1.24±0.16	0.299	**0.029**	0.82±0.08	1.94±0.31	**0.015**	**0.034**

Creatinine (µmol/l) Mean±SEM	100.24±5.54	102.54±5.40	0.614	0.578	87.36±6.61	106.63±8.05	*0.072*	0.511
GFR (ml/min/1.73m²) Mean±SEM	64±5.54	69.97±4.05	0.620	0.134	77.10±6.80	65.50±5.70	0.361	0.483

4.6.4. Investigating the role of single nucleotide polymorphism L55M in the gene of PON1 as risk factor and its influence on the survival of the patients with ACS;

No associations were found between 1-year survival and PON1 L55M genotypes, neither in the entire group of patients (p=0.640) nor in the subgroups with different diagnoses (p=0.902 for STEMI; p=0.108 for UA+NSTEMI).

After 5 years of follow-up, the number of patients who are alive is in total 49 (63.6% survival). No associations have been observed with PON1 SNP (p=0.561).

The same lack of association of PON1 SNP (p=0.389) is identified after 9 years of follow-up of the patients when the percentage of survival is 50.6% (39 of 77).

The Kaplan-Meier survival curve after one year of follow-up shows that, although not significantly, patients with the LL genotype have an unfavorable prognosis (average period of survival of 17.81 months) in comparison with patients with M allele genotypes (LM+ MM), average period of survival of 20.31 months, p=0.553, Log rank test) (Figure 21A). This difference is expressed more in patients with NSTEACS (p=0.115) (Figure 21C).
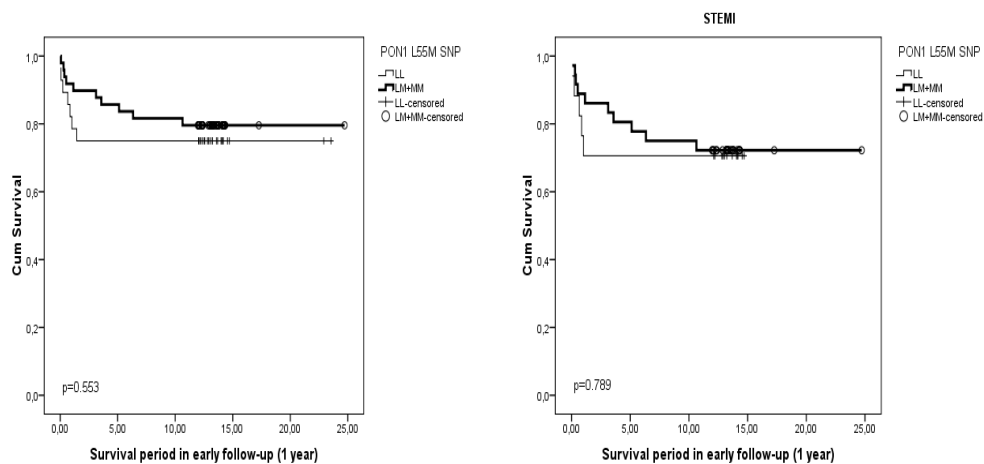
Figure 21. A.

Figure 21. B.
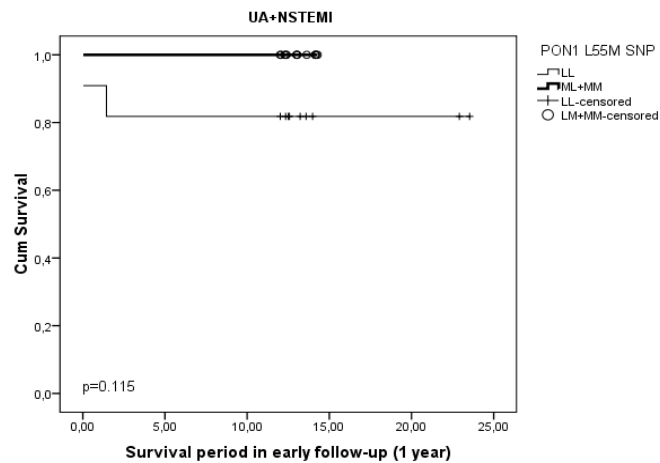
Figure 21.C.

Figure 21. Kaplan-Meier survival curves of patients with different PON1 L55M genotypes and diagnosis after 1 year of follow-up. 21A - the entire group with ACS; 21B - patients with STEMI; 21C - patients with NSTEACS.

In the average period of 5 years (60 months) of follow-up, the visible difference between the patients with LL and those with M allele genotypes (LM_MM) is completely lost (Figure 22A). Even in the longer period of 9 years (108 months) of follow-up, although insignificantly, the prognosis for the patients with LL genotype becomes more favorable than the prognosis for the patients with LM or MM genotypes (Figure 22B), especially for the patients with NSTEACS (Figure 22C).
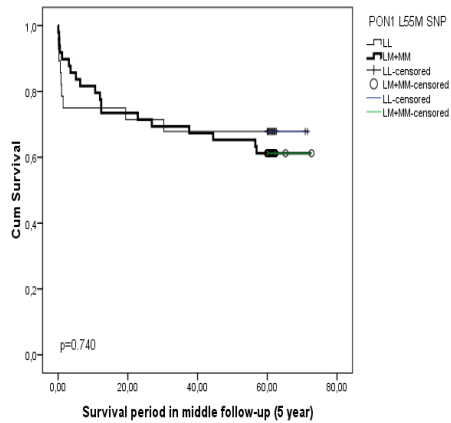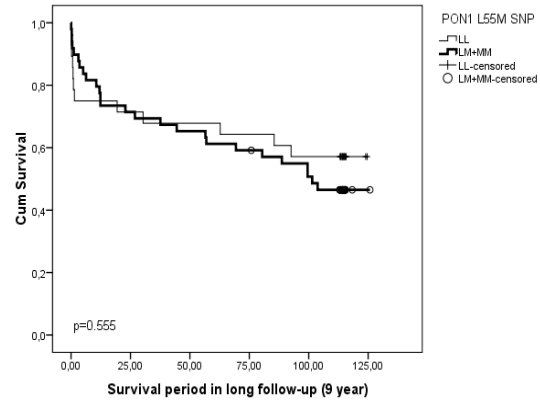
Figure 22.A.   *Figure 22.B.*
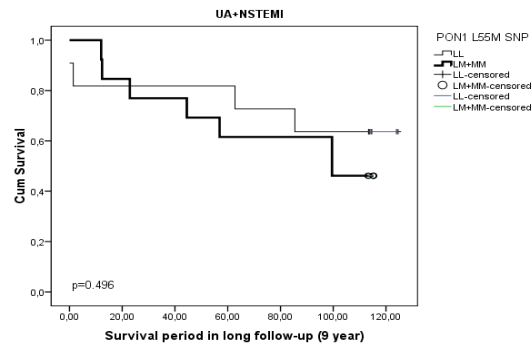
Figure 22.C.

Figure 22. Kaplan-Meier survival curves for 5 years (22A) and 9 years (22B) follow-up of the patients of the entire group with ACS, but with different PON1 L55M genotypes. 22C - Kaplan-Meier survival curves for 9 years follow-up of patients with NSTEACS, but with different PON1 L55M genotypes.

4.7. Survival of patients with ACS. Developing a prognostic model of the 1st, 5th, and 9th-year survival.

4.7.1. Survival of patients with ACS;

The cumulative average survival of patients according to the diagnosis is presented in the table below 10.

Table 10: Total survival rate of patients based on diagnosis at the 1st year.

Overall survival until first year (12 months)				
Diagnosis	Total number	Deceased	Alive	
			Number	%
STEMI	102	24	77	76.2
UA	43	3	41	93.2
NSTEMI	25	4	21	84.0
Total	170	31	139	81.8

Figure 23 represents the total average survival curve in patients in the 1st and 3rd year, and Figure 24 represents the survival curve of the patients based on diagnosis in the first and third year. The overall survival curve shows a decline until the 12th month (88% survived individuals), after that, a plateau occurs until the 24th month, and then it deteriorates again with a pronounced dip towards the 36th month (3rd year). The cumulative survival for all patients until the 2nd year is 85%, and in the 3rd year - 82%.
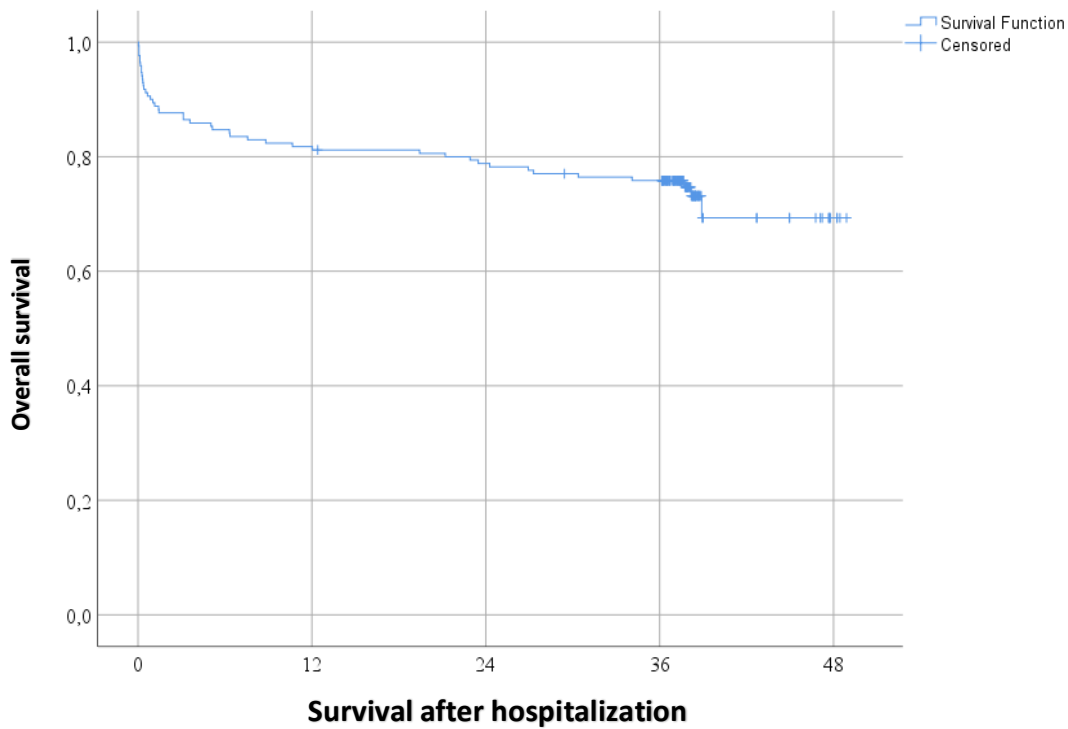
Figure 23: Kaplan-Meier curve for the cumulative survival of patients until the 3rd year.
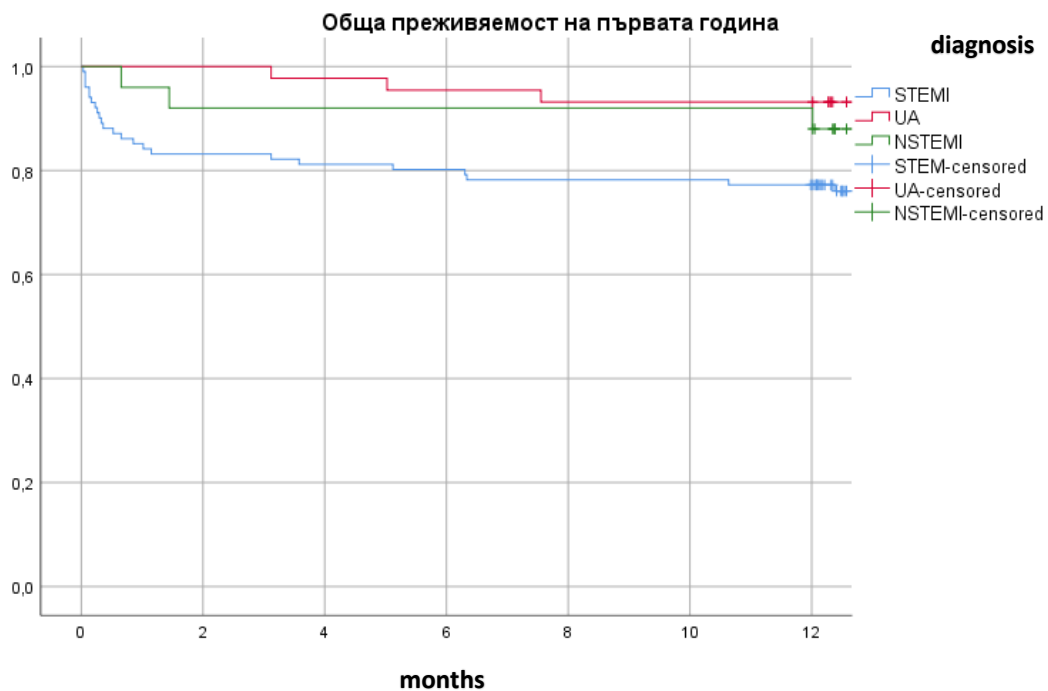
Figure 24: Kaplan-Meier curve for the cumulative survival of patients in three diagnoses in the 1st year by diagnosis (STEMI, NSTEMI, UA).

The analysis the survival curve in individuals with STEMI during the follow-up shows the decline of the survival rate until the 6th month, after that a plateau occurs until the 24th month with small fluctuations and followed by significant decline after the 36th month (blue line). Similar trend of the curve appears in NSTEMI with less fluctuations and with approximately constant survival rate after 24th month (green line). The most favorable cumulative survival rate is observed in the curve of individuals with UA (red line). After the 3rd year of the follow-up there is a significant divergence in survival rates among individuals with STEMI and UA (p=0.012).

The overall cumulative mortality rate among the patients is 28.2% at the fifth year, while the survivors account for 71.8% (Table 11).

Table 11: Overall survival of patients based on diagnosis in the 5th year.

Overall survival rate up to the fifth year (60 month)				
Diagnosis	Total number	Number	Survived	
		Non-survived	Number	%
STEMI	102	33	68	67.3%
UA	43	7	37	84.1%
NSTEMI	25	8	17	68.0%
Total	170	48	122	71.8%

At the ninth year of follow-up, the overall mortality rate is 39.2% (67), while the survivors account for 61.2% (104). The average survival after hospitalization as of August 1, 2019, is 88.7 months. The survival rate by diagnosis at the ninth year (108 months) is shown in Table 12.

Table 12: Overall survival of patients based on diagnosis in the 9th year.

Overall survival up to the 9th year (108 months)				
Diagnosis	Total number	Number of deceased	Survived	
			Number	%
STEMI	102	47	55	53.9%
UA	43	11	32	74.4%
NSTEMI	25	10	15	60.0%
Total	170	68	102	60.0%

The overall survival curves for the three diagnoses at the fifth year demonstrate the following: a slight fluctuation with a decrease in survival after the third year and almost constant survival after the 40th month in the unstable angina group (red line), gradually decreasing survival in the NSTEMI group (green line), and constant survival with small fluctuations towards the 40th month in the STEMI group (blue line). Around the fifth year, the overall survival curves for NSTEMI and STEMI intersect, and the cumulative survival coincides (Figure 25B).

Overall survival after the fifth year
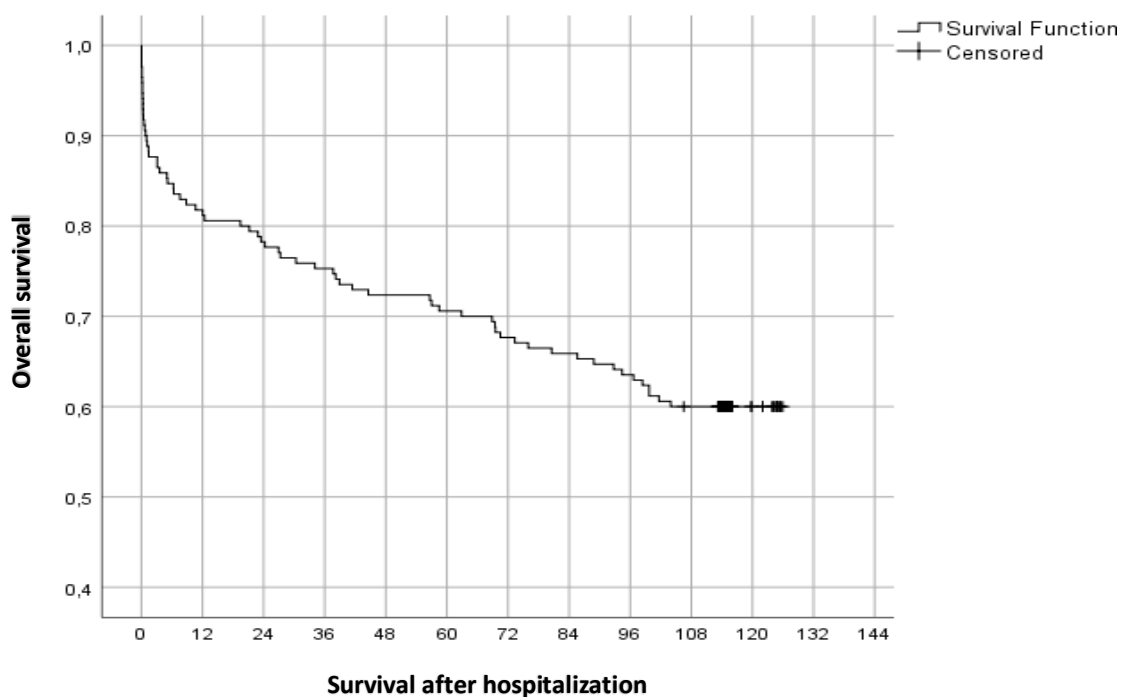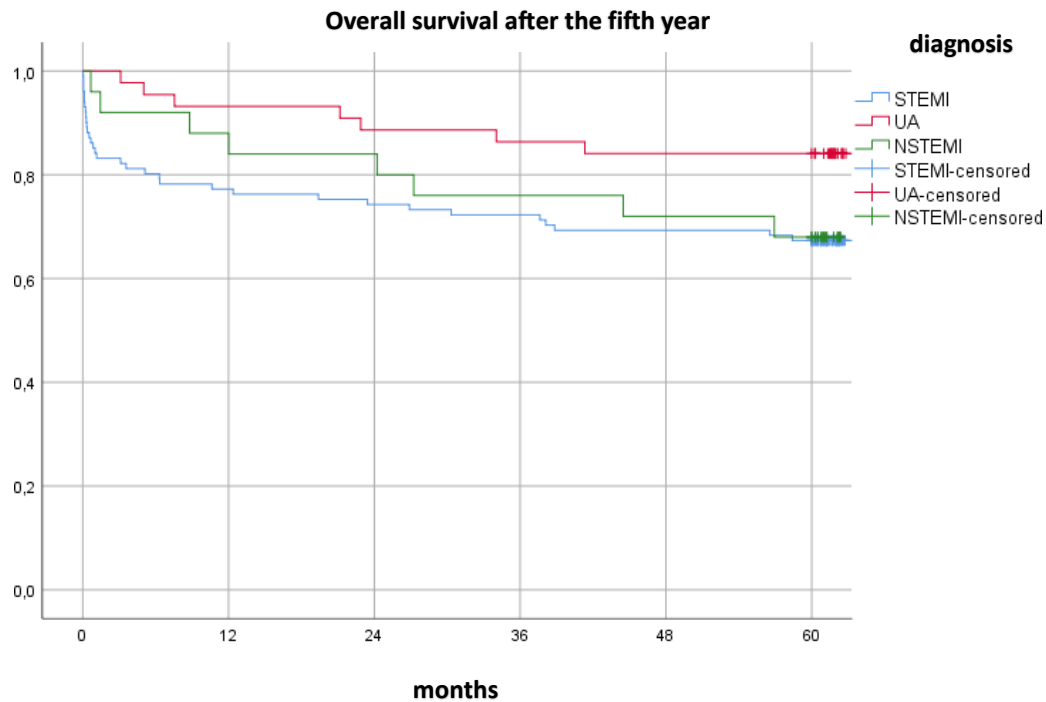
Figure 25: Kaplan-Meier's curve for cumulative average survival of all patients (A) and for the three diagnoses (STEMI, NSTEMI, UA) up to the fifth year (B).

In Figure 26A and 26B below, the overall survival of patients at the ninth year and by diagnosis during the follow-up is presented. The curve of the 9-year overall survival for the entire group (Figure 25A) shows a steep downward trend after hospitalization, indicating an increasing cumulative mortality rate with the emergence of a constant trend around the 12th and again around the 48th month. Subsequently, there is a continued increase in overall mortality with smaller fluctuations, reaching 60% at 108 months, after which it shows a constant trend (Figure 26A).

The Kaplan-Meier curves at the ninth year for NSTEMI and STEMI intersect, indicating a worsening of survival in the latter group, while the curve for UA shows small fluctuations (Figure 26B).

The Cox regression analysis revealed a significantly higher survival rate among patients diagnosed with UA compared to those diagnosed with STEMI at

the ninth year (p=0.028), while no significant difference in survival was found between STEMI and NSTEMI.

A

Overall survival after the ninth year

B

months
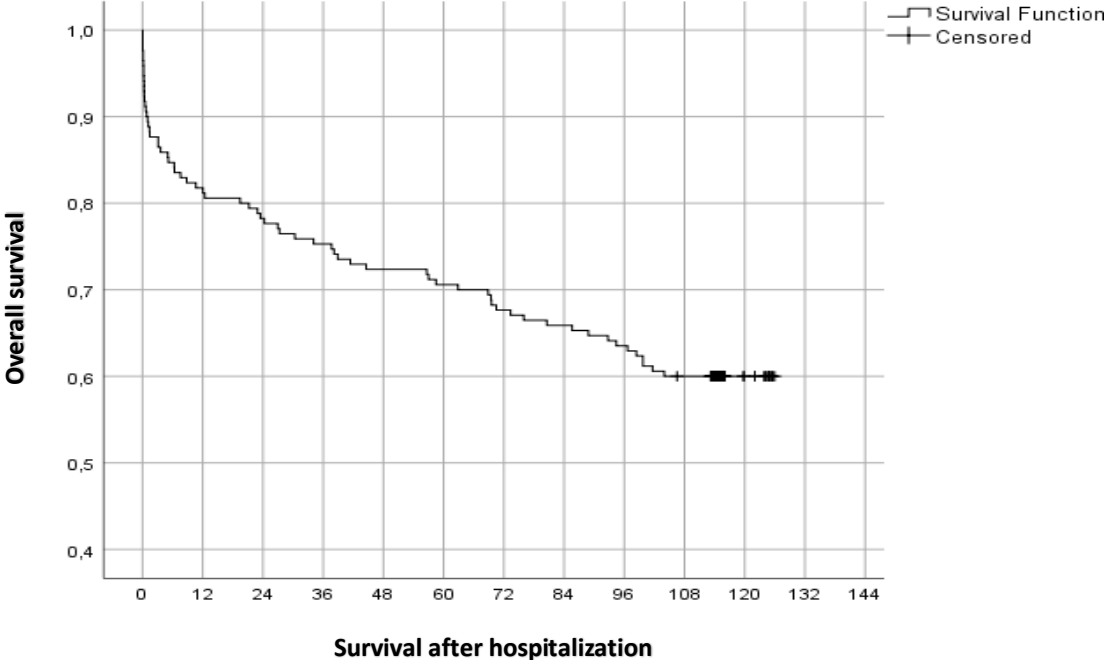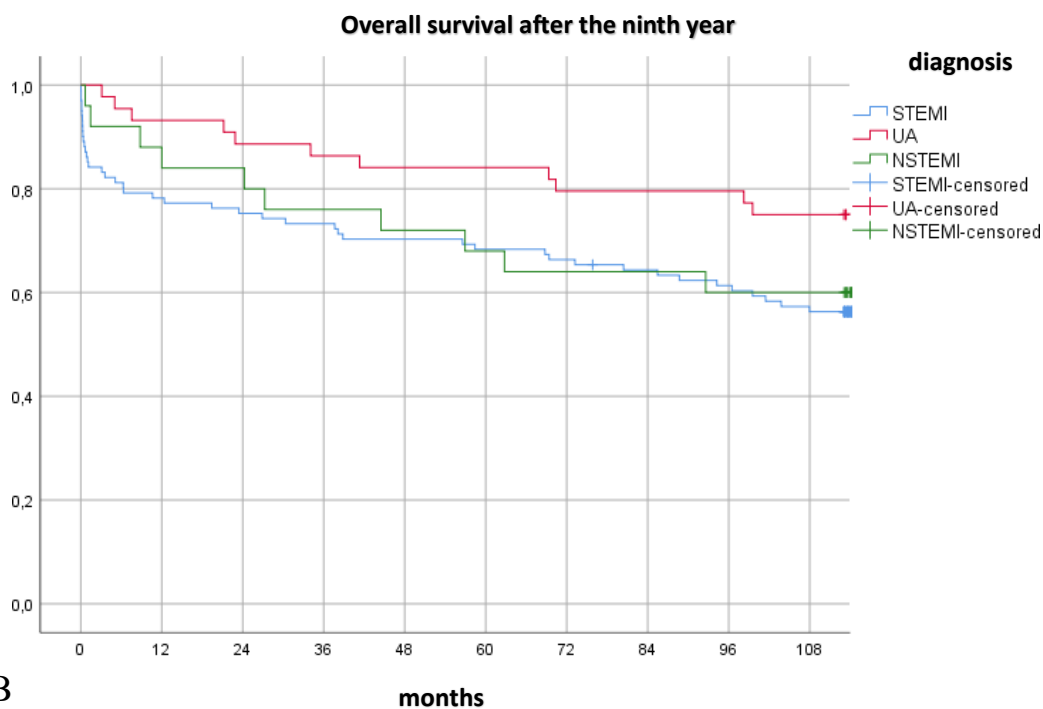
Figure 26: Kaplan-Meier curve for cumulative average survival of all patients (A) and for the three diagnoses (STEMI, NSTEMI, UA) up to the ninth year (B).

By dividing the patients according to the classification of the World Health Organization (WHO), we obtained the data presented in Table 13.

Table 13. Distribution of the patients by age according to WHO.

Age(years)	Number	Deceased	Survived		Average survival
			Number	Percentage	(95% DI) months
Young <45	9	1	8	88,9	103.3(80.2; 126.4)
Older age (45-59)	66	12	54	81,8	107.9(98.4; 117.5)
Adults (60-74)	64	31	33	51,6	83.3(71.1; 95.6)
Old (75-89)	31	21	10	32,3	62.5(44.0; 81.0)

| Total | 170 | 65 | 105 | 61,8 | 90.9(83.5; 98.4) |

From the provided data in Table 13, it can be observed that individuals in the older age group have the highest overall survival, with a significant decline in months within the age range of 60-74 years. Individuals above 75 years old have the shortest duration. It is worth noting that younger individuals, although not significantly, have lower survival compared to those in the age group of 45-59 years. Logically, older individuals have the shortest average duration, approximately 62.5 months.
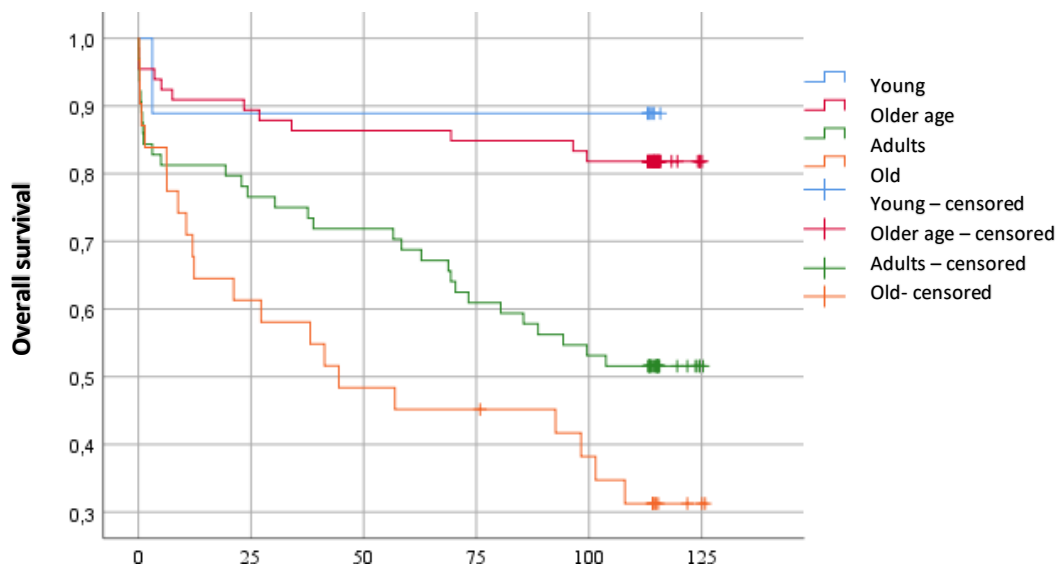
Figure 27: Kaplan Maier's curve for survival of patients based on age according to WHO.

From the above-presented curve, it is evident that patients in young age have significantly longer survival compared to those in old age (p=0.009). Individuals in older age outlive those in the age range of 60-74 years, indicating a higher survival rate (p=0.009;p<0. 001) (Figure 27).

Analyzing the survival rate of the patients with/without diabetes mellitus, we discovered significantly longer survival in individuals without diabetes mellitus (p=0.009). This dependency is observed throughout the 9-year observation period in the studied group of individuals after CABG surgery (Figure 28).
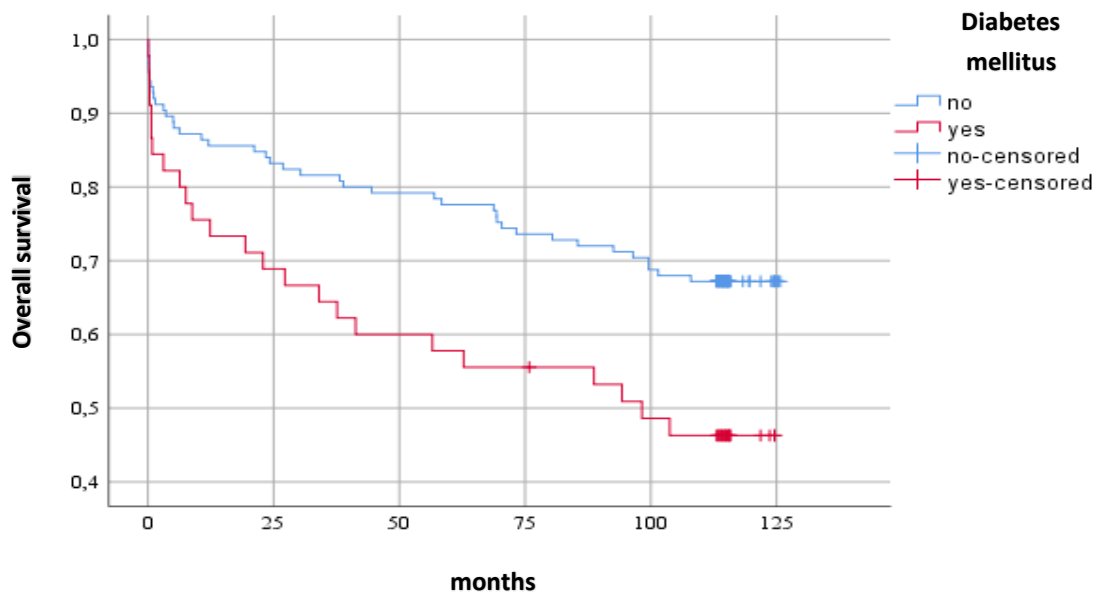
Figure 28: Kaplan-Meier curve of the survival rate of patients with ACS depending on the presence of diabetes mellitus (DM) (p=0.009).

Similar dependency we discovered in presence of the smoking risk factor among the examined individuals, presented on Figure 29.
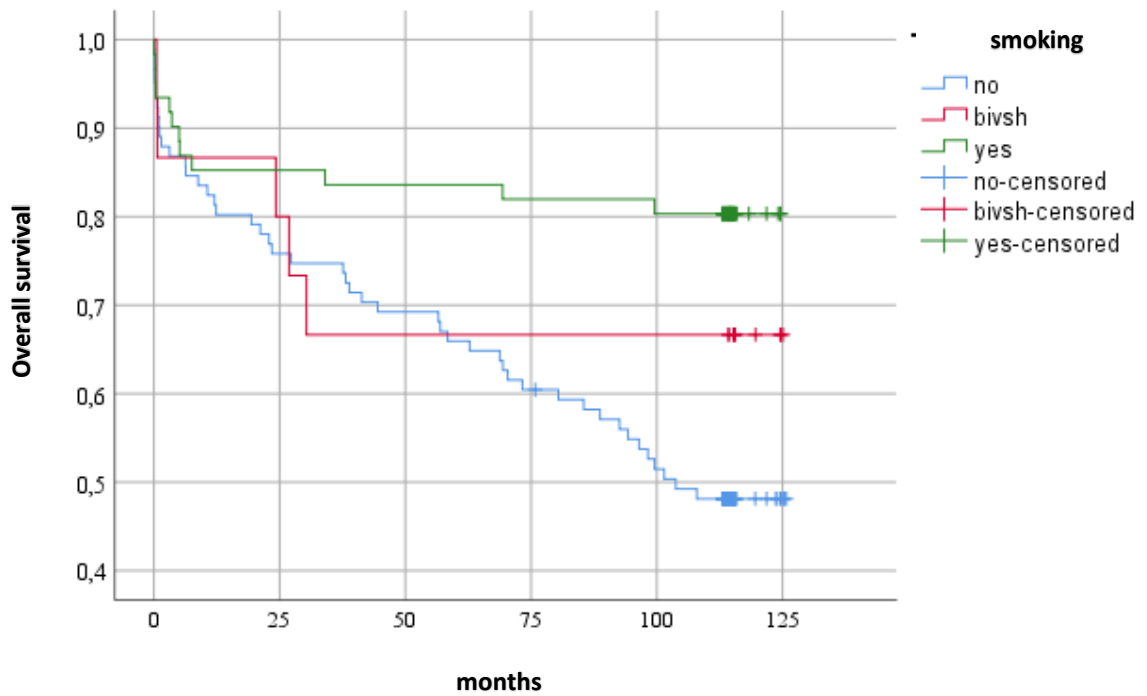
Figure 29: Kaplan-Meier curve of the survival rate of patients with ACS depending on the presence of the risk factor – smoking (p<0.001).

We observed a significantly shorter lifespan after ACS among smokers (p<0.001) (Figure 29). The ex-smokers had a longer survival rate than the current smokers, however, even on the ninth year of follow-up, the survival rate of smokers did not reach that of non-smokers.

Analyzing the glomerular filtration (GFR) in the risk constellation for survival after ACS, we distributed the GFR based on the classification of the International Society of Nephrology is presented in Table 14.

Table 14: Average survival rate of patients based on the value of their glomerular filtration (GFR).

			Survived		Average survival rate
				Percentage	(95% DI) months
GFR ml/min/1.73m2	Number	Deceased	Number	(%)	
<30	5	5	0	0.0	6.5(0.0; 15.4)
30-44	21	18	3	14.3	47.9(29.2; 66.6)

45-59	29	13	16	55.2	84.4(65.8; 103.0)
60-89	71	19	52	73.2	103.4(93.8; 113.0)
>=90	42	10	32	76.2	102.5(89.2; 115.9)
Total	168	65	103	61.3	90.5(83.0; 98.1)

It is evident that individuals with advanced kidney disease have the lowest survival rate (IV and V stage) with GFR < 30ml/min/1.73m. Obviously, the longest lifespan after ACS in our group had those with stage I and II of renal disease (Б3). The survival rate during the nine years of observation is shown on Figure 30.
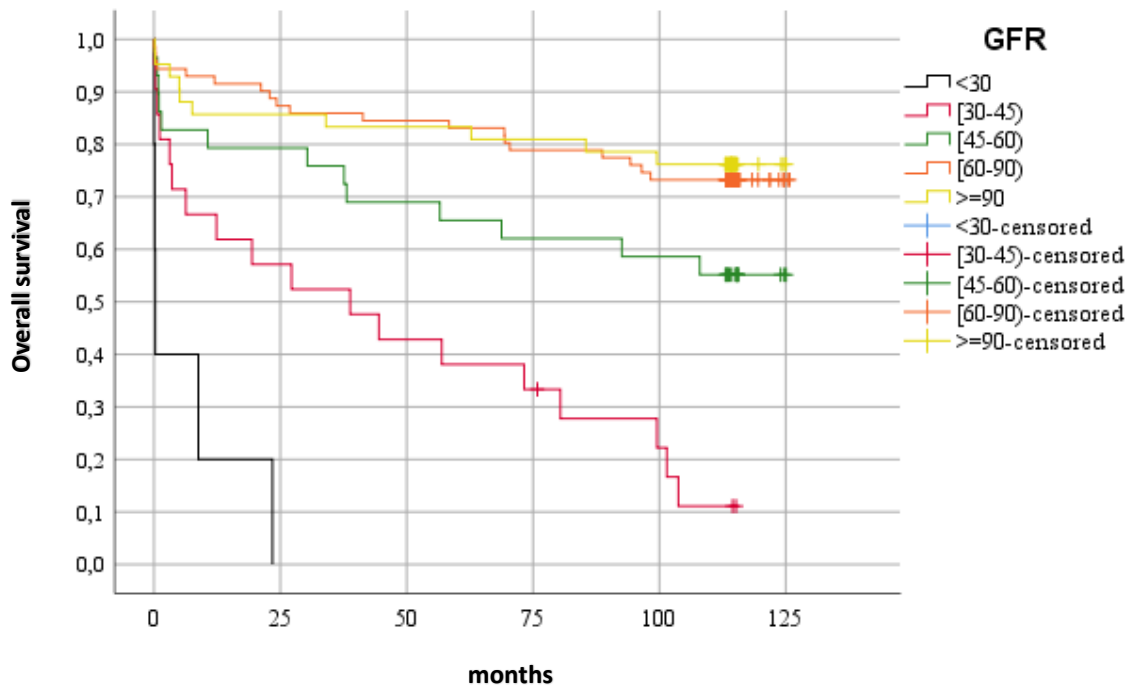
Figure 30: Kaplan-Meier survival curve of patients until the 9th year based on the glomerular filtration (GFR).

The individuals with renal disease stage IV and V have significantly shorter survival than all other groups (p<0.001). The patients with stage III renal disease, whose GFR is within the range 30-45 ml/min, have significantly survival than those with GFR within the range 45-59 ml/min/m² (p=0.003) and especially than the individuals with GFR within the range 60-89 ml/min/m² (p<0.001) and >90

ml/min/m^2 (p<0.001). The individuals with stage II renal disease with GFR within the range 45-59 ml/min/m^2 have a tendency for shorter lifespan than the groups with GFR between 60-89 ml/min/m^2 (p=0.063) and >=90 ml/min/m^2 (p=0.067). No difference was found in the survival rate of individuals with stage I and II of renal disease.

The dyslipidemia as a risk factor did not demonstrate significant impact on the survival of individuals in the 1st and 5th year of the follow-up (p=0.107) (p=0.169), but in the 9th year an adverse prognosis was observed (p=0.073) (Figure 31).
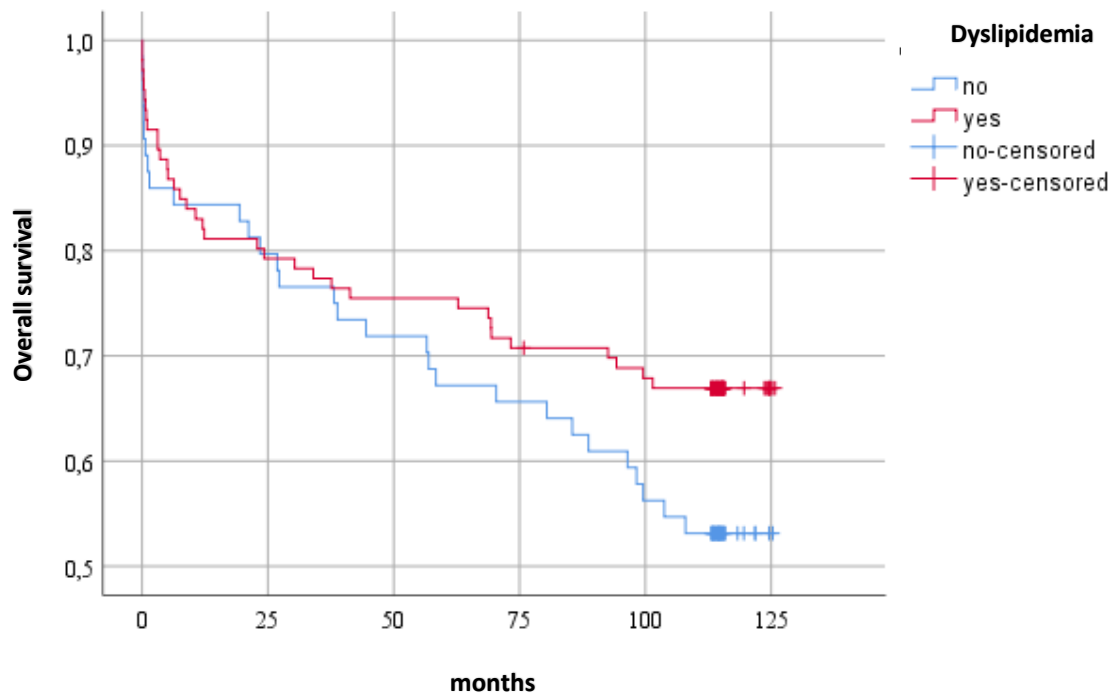
Figure 31: Kaplan-Meier survival curve of patients with ACS based on the presence of the risk factor - dyslipidemia.

Patients with ejection fraction of LSV under 40% (EF<40%) have significantly worsened survival curve already at the time of hospitalization, which continues to worsen until the 2nd year, and at the 3rd year a plateau occurs (p=0.006, p=0. 006, p=0. 015) in comparison with the remaining patients with EF>40%, regardless of the diagnosis (Figure 32).
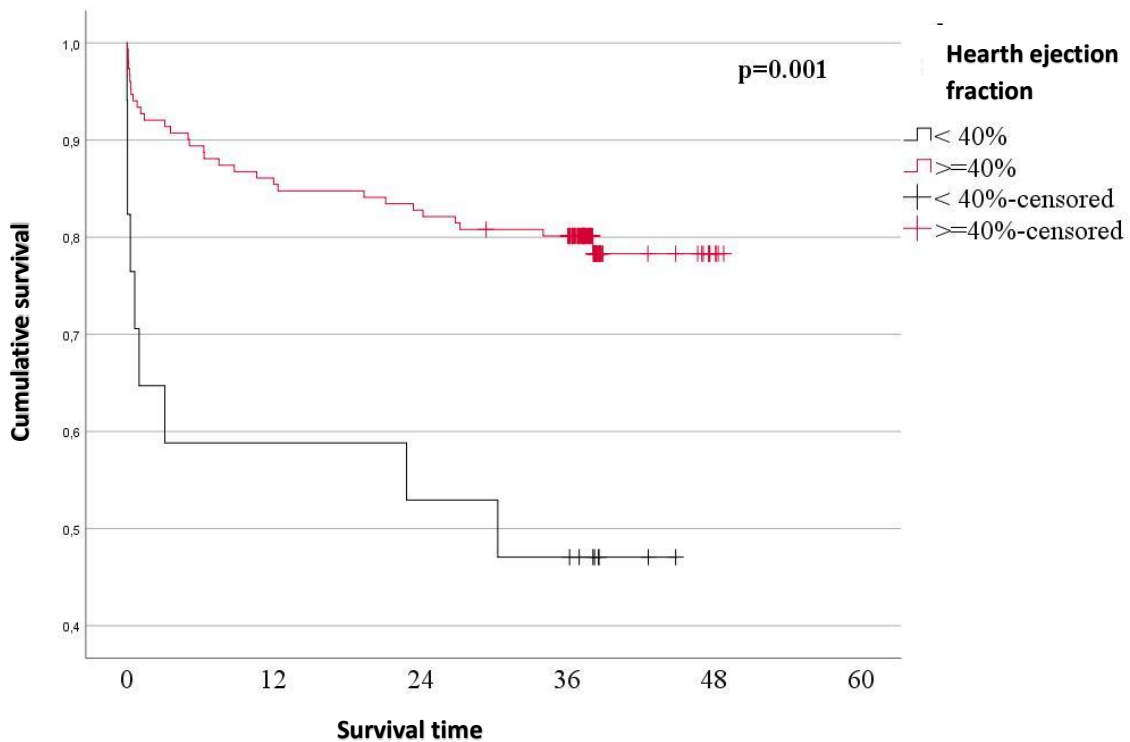
Figure 32: Kaplan-Meier curve of overall survival of patients with ejection fraction (EF) (<40% vs. >40%) for a period of 48 months.

At the 5-year follow-up, patients who had an ejection fraction (EF) of less than 40% at the time of hospitalization maintained an unfavorable overall survival. At the same stage of the follow-up the individuals with known three-vessel coronary disease have a tendency for worse survival (p = 0.001, p=0.028) in comparison with those without such disease (Figure 33).
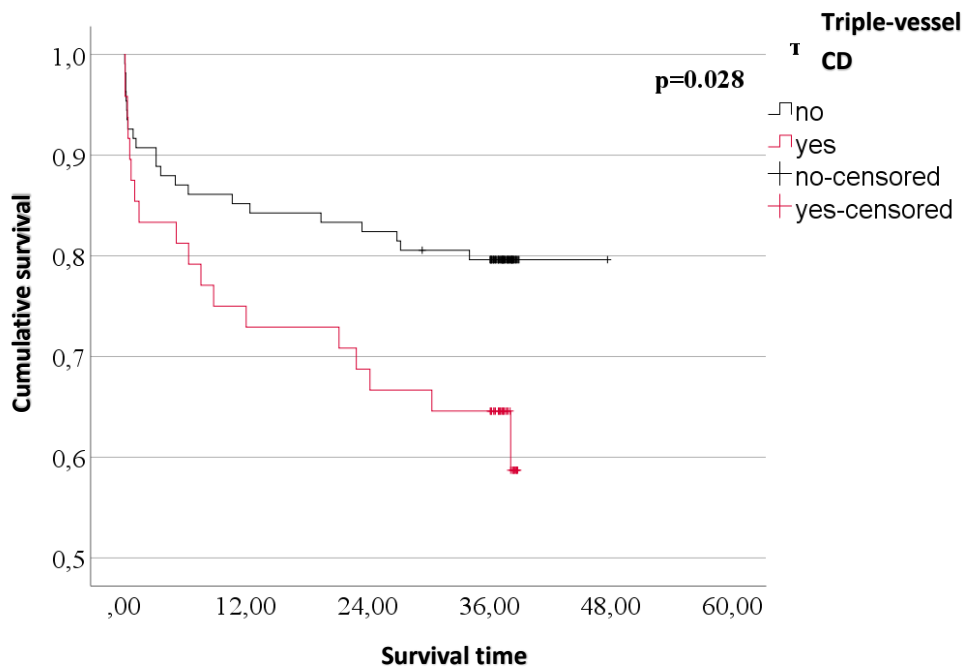
Figure 33: Kaplan-Meier curve of overall survival of patients with triple-vessel coronary disease (CAD) until 48th month.

At the ninth year of follow-up, the overall mortality rate is 39.2% (67), while the survivors account for 61.2% (104). The average survival after hospitalization as of August 1, 2019, is 88.7 months. The diagnosis-specific survival at the ninth year (108 months) is presented in Table 12.

» Determining the factors for mortality in patients with ACS in the first year.

In conducting a one-way analysis, we found that significant adverse prognostic factors for survival up to the first year are diagnosis, age, and glomerular filtration rate (GFR), GRACE – score >140 p., presence of ALSHF>II Killip at hospitalization, experienced acute MI and ejection fraction of LV (EF<40%) (Table 15).

Table 15. One-way logistic regression for the first year. Dependent variable - mortality. Independent variables – potential factors for mortality.

Factor	(p-value)	OR (95% CI)
Diagnosis	0.029	

STEMI		1
UAP	**0.024**	**4.260 (1.210; 14.997)**
NSTEMI	0.099	3.584 (0.787; 16.320)
Age	**0.011**	**0.954 (0.920;0.989)**
Glomerular filtration (ml/min)	**0.012**	
<60		1
60-90	**0.003**	**5.289(1.764; 15.859)**
>90	0.245	1.833(0.659; 5.099)
Grace score	**<0.001**	
<=140		1
>140		**0.147 (0.060; 0.357)**
Killip>II	**<0.001**	
No		1
Yes		**0.162 (0.058; 0.491)**
Experienced acute MI (EAMI)	**0.039**	
No		1
Yes		**0.379 (0.151; 0.953)**
Ejection fraction (EF, %)	**0.016**	
< 40		**1**
40-49	**0.041**	**3.500(1.052; 11.645)**
>50	**0.004**	**5.444 (1.710; 17.336)**

» Determining the factors for mortality in patients with ACS at the 5th year.

The results of the univariate analysis for 5-year survival after hospitalization confirm the same prognostic factors, along with the additional presence of two factors - smoking and diabetes mellitus. The presence of these two additional risk factors is associated with a more unfavorable outcome (Table 16).

Table 16. One-way logistic regression for the 5th year. Dependent variable – mortality. Independent variables – potential factors for mortality.

Factor	(p-value)	OR (95% CI)
Diagnosis	0.119	
STEMI		1
UAP	**0.042**	**0.390 (0.157;0.967)**
NSTEMI	0.949	0.970 (0.380; 2.476)
Age	**<0.001**	**0.940 (0.911; 0.970)**
Dyslipidemia	0.169	
No		1
Yes		0.620 (0.314; 1.224)

Glomerular filtration (ml/min)	**<0.001**	
<60.0		1
60-90	**<0.001**	**0.213 (0.093; 0.489)**
>90.0	**0.003**	**0.230 (0.086; 0.616)**
Smoking	0.043	
Smoker		1
Ex-smoker	0.890	0.922 (0.290; 2.931)
Non-smoker	**0.013**	**0.362 (0.162; 0.807)**
Grace score	**<0.001**	
<=140		**1**
>140		**7.208(3.098; 16.770)**
Killip>II	**0.004**	
No		1
Yes		**4.316(1.612; 11.557)**
Diabetes mellitus	**0.017**	
No		**1**
Yes		**2.419 (1.174; 4.984)**
Experience acute MI (EAMI)	<0.001	
No		1
Yes		**4.597 (2.023; 10.446)**
Ejection fraction (EF, %)	**0.003**	
< 40		**1**
40-49	**0.037**	0.292 (0.092; 0.930)
>50	**0.001**	**0.148(0.048; 0.458)**

» Determining the independent factors for death in patients with ACS at the 9 th year.

The one-way logical analysis at the 9 th year showed that the presence of smoking, DM, experienced AMI, CVD for bad prognostic factors for survival. The impact is similar on the survival with advancing age and the presence of high GRACE-score (>140 p.), and the normal renal function (GFR>90 ml/min) and the lack of signs of heart failure (ALVHF) at hospitalization have favorable (protective) effect on the prognosis for the patients in 9-year survival (Table 17).

Таблица 17. Univariate analysis to assess the effect of various demographic, clinical, and biochemical variables on the 9-year survival of patients.

Factor	(p-value)	OR (95% CI)
Diagnosis	0.112	
STEMI		

NAP	**0.037**	**0.432 (0.196; 0.949)**
NSTEMI	0.747	0.864 (0.354; 2.106)
Age	**<0.001**	**0.920(0.891; 0.951)**
Dyslipidemia	0.073	
No		1
Yea		0.559 (0.296; 1.056)
Glomerular filtration (ml/min)	**<0.001**	
<60.0		1
60-90	**<0.001**	**5.053 (2.319; 11.011)**
>90.0	**<0.001**	**5.333 (2.126; 13.377)**
Smoking	**<0.001**	
Smoker		1
Ex-smoker	0.171	2.233 (0.707; 7.049)
Non-smoker	**0.008**	**4.558 (2.145; 9.684)**
Grace score	**<0.001**	
<=140		**1**
>140		**0.126(0.051; 0.316)**
Killip>II	**0.008**	
Не		1
Да		**0.247 (0.089; 0.689)**
Diabetes mellitus	**0.008**	
No		1
Yes		**0.390 (0.195; 0.784)**
Experienced acute MI (EAMI)	**0.001**	
No		1
Yes		**0.260(0.114; 0.590)**
Ejection fraction (EF,%)	**0.002**	
< 40		1
40-49	0.171	2.281 (0.700; 7.439)
> 50	**0.003**	**5.622(1.786; 17.702)**
Cerebro-vascular disease (CVD)	0.053	
No		1
Yes		0.324 (0.103; 1.015)
Anemia	**0.027**	
No		**1**
Yes		**0.328(0.122; 0.883)**

Of all the analyzed factors that have a significant impact on survival, only the GRACE score is identified as an independent predictor of mortality, as determined through multivariate regression analysis in the study (p = 0.002, OR=1.052).

Through univariate logistic regression and stepwise procedure, we identified the strongest independent factors for mortality and created models for determining the probability/chance of an adverse event (death) in each patient. The models for predicting the probability/chance of an adverse event (death) at the 1 st , 5 th and 9 th year that we received are:

$$p_{\text{модел\_}1} = \frac{e^{4.061+\ 1.475*GFR}}{1+\ e^{4.061+\ 1.475*GFR}}$$

$$p_{model\_5} = \frac{e^{5.621-\ 0.079*Age+1.831*GFR^{*}-1.197*DM-1.905*EAMI}}{1+\ e^{5.621-\ 0.079*Age+1.831*GFR^{*}-1.197*DM-1.905*EAMI}}$$

$$p_{model\_9} = \frac{e^{5.356-0.090*Age-1.490*GFR^{*}-1.694*EAMI}}{1+\ e^{5.356-0.090*Age-1.490*GFR^{*}-1.694*EAMI}}$$

where **p** is the probability of a patient surviving beyond 12th, 60th and 120th month from study enrollment.

5. Consideration

The main objective of this study was to investigate certain clinical, instrumental, biochemical, and genetic markers that contribute to the risk profile of patients with ACS and analyze their contribution to the short-term and long-term prognosis of this patient group.

Men are hospitalized for acute coronary syndrome (ACS) as an emergency 1.5 times more often than women, which is confirmed by other studies as well. (Gotto et all.,2007). In our study the average age of the patients is 61.5±11.9, which is close to the data from published studies (Zhong Z. et all., 2017). In the subgroup analysis by diagnosis the youngest are the patients in the group of UA(60.2±10.7(39.0;86.0), followed by STEMI(60.9±12.1(32.0;85.0) and the oldest are the patients with NSTEMI 66.2±12.3(42.0;86.0), and the patients with STEMI are significanly younger than those with NSTEMI (Santos I.,2015). The statement regarding women, who on average experience acute coronary events approximately 4 years later than men, is confirmed.

Task 2. The distribution of modifiable risk factors in our study group closely resembles the profile of patients in other published studies (González-Pacheco et all, 2014). Arterial hypertension is one of the key triggering mechanisms leading to accelerated atherogenesis with the development of unstable and vulnerable plaques, which are responsible for the onset of acute coronary syndrome. In the general population, the prevalence of arterial hypertension increases linearly with age in both genders. The authors of SYMPHONY report a presence of AH in over 50% of the participants, and the Spanish register reports for 46% prevalence of hypertension among patients with STEMI. Unlike the previously mentioned studies, in our research, the most common risk factor is a history of hypertension, which is present in 81.4% of the cases and with no significant difference in genders and by diagnosis. In the group of patients with STEMI hypertension is prevalent among 82.52%, NSTEMI - 84%, UAP - 88.64%. Out of those with hypertension, 80.2% reported having a known diagnosis of hypertension. Regarding the level of control, 56.7% reported good control of hypertension, while 43.4% reported poor control. In our data, there is no difference in the frequency of hypertension prevalence between ST-elevation myocardial infarction (STEMI) and non-ST-elevation acute coronary

syndrome (NSTE-ACS). This is most likely due to the small number of patients with unstable angina and non-ST-elevation myocardial infarction, which is a limitation of the study. A subgroup analysis identified that the patients with known AH are 80.2%, and 19.8% with newly discovered AH, which confirms again its significantly higher frequency from the published data. The frequency of diabetes among our study group is 25.4%, with no difference between genders, which again is higher than the reported rates (Anand S., 2008, Zhong Z., 2017). The data from Romanian registries partially align with the established risk profile of our patients: 67% have arterial hypertension, 24% have diabetes, 41% have lipid disorders, 39% are smokers, and 8% have a history of myocardial infarction (Cretu D., 2015). There is a significantly higher frequency of AH, diabetes mellitus among women, and a higher percentage of smokers among men. In the study by Gonzalez-Pacheco et al., the most prevalent risk factors were smoking (68%), dyslipidemia (47.5%), and diabetes (37.7 In terms of gender distribution, the most common risk factors among women are arterial hypertension (AH), diabetes (DM), and dyslipidemia, while smoking is the leading risk factor among men. According to the same author's collective, the number of women with 2 or more risk factors was significantly higher. In the Swiss registry AMIS, women were older than men and had a higher prevalence of hypertension, dyslipidemia, and diabetes, but a lower prevalence of overweight or smoking (Radovanovic D., 2007). The analyses from the current study show that among hospitalized patients, the age of women is significantly higher than that of men, but arterial hypertension (AH), dyslipidemia, and smoking are more common among males. The prevalence of dyslipidemia, diabetes mellitus, and family history is consistent with the published data in the literature (Liu K., 2021). The significantly higher proportion of smoking among men is also consistent with the findings (Radovanovic D., 2007, Dali B., 2014). Based on our own data and the literature, the high percentage of AH and dyslipidemia in our group may be associated with the higher frequency of diabetes mellitus. The development of diabetes is

significantly influenced by AH, overweight, elevated triglyceride levels, and low HDL-C levels, despite the small number of patients in the study. This could possibly be explained by socioeconomic factors and lifestyle, which have not been investigated in the current study (Dali B., 2014).

Analyzing the factor of time from symptom onset to hospital admission, we did not obtain data supporting its association with patient prognosis. Most likely, this is related to the time interval of the study initiation back in 2009 when the awareness among the Bulgarian population was lower. The largest percentage of patients sought medical assistance after 24 hours from the onset of chest pain (n=96, 55.8%). A relatively small number of patients with STEMI (32, 31.1%) sought medical assistance up to the 6th hour from the onset of the symptomatic. We did not find an association between the time of the onset of the clinical symptomatic and the overall survival in the hospital at 6th, 12th, 24th and 108th month. Timely or early seeking of medical help is not related to gender and age in our study population (Liu K., 2021).

Analyzing the correlation of the serum values of the lipids with the diagnosis we identified only higher values of TC and LDL-C in patients with ACS with ST-elevation, which is known from other authors (Zhong Z., 2017., Tai S., 2020). We identified that men in our group have lower values of TC, LDL-C, HDL-C and TG than women without reaching considerable difference. The correlative analysis between the indicators of the lipid profile and the age shows interesting and unexpected results: weak positive correlation with HDL-C and weak negative correlation with TG. These correlations are lost among the women's group with advancing age, which probably can be explained with gender-specific characteristics, while in men it remains significant (Tai S., 2020). Regarding the total cholesterol and HDL-C a descending gradation is observed. For HDL-C and TG we obtained statistically significant difference of the levels in the different age groups, and significantly lowest levels of HDL-C are observed in young men under 49 years, while the adults over 70 years have significantly

highest TG levels towards the other age groups. This was not confirmed in the age groups among women. This distribution of HDL-C levels varies according to ethnicity and gender, as observed in other studies (Davis CE., 1996, Numasawa Y., 2015). The higher levels of HDL-C in women partly explain the lower frequency of ACS and later onset of ACS, which supports the hypothesis that having comparable levels in men could potentially reduce and delay the progression of IHD among males (Davis CE., 1996). Overweight and obese patients are more likely to suffer from cardiovascular diseases, particularly ACS (Colombo M., 2015). The literature contains data on the correlation between values of total cholesterol (TC) and LDL cholesterol (LDL-C) with BMI, which we did not observe in our study. Our data showed the expected positive association between triglycerides (TG) and BMI, and a negative association between HDL cholesterol (HDL-C) and BMI, confirming the well-known fact that individuals with overweight and obesity have lower levels of HDL-C (Feder D., 2002). In terms of diabetes mellitus, we obtained contradictory results, in the individuals with diabetes there is a tendency for lower values of TC, significantly lower levels of LDL-C and higher levels of TG compared with the individuals without diabetes mellitus, which is well-known and has been confirmed. This is likely due to the intake of lipid-lowering medications among individuals with DM, which our study cannot explain with the available data, which we note as a deficiency. The HDL-C was higher in individuals without diabetes, with no presence of statistical significance. (Colombo M., 2015). In the present study, the group with diabetes included a higher proportion of individuals with overweight and obesity compared to non-diabetics, which is consistent with findings reported by several other authors (Gu K., 1999, Colombo M., 2015). In the literature, it is a well-known fact, and there are many published data on the association of AH with levels of blood glucose (BG), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C), which were not confirmed in our results (Laurenzi M., 1990). It is known that a family history of early IHD is an independent risk factor

for SSDM and the identification of individuals at risk and timely primary prevention could prevent future events (Roncaglioni MC., 1992). We did not find correlation between the family history for early IHD and the examined biochemical indicators of the lipid profile in our group. When analyzing these correlations in both genders it was identified that TG are significantly higher in women with family history than in those without family history. By analyzing the correlation between serum creatinine and glomerular filtration rate (GFR) with demographic and risk factors, we confirmed the findings of other authors regarding the significance of chronic kidney disease (CKD) as a risk factor for ischemic heart disease (IHD). Patients with lowest glomerular filtration are in the group with STEMI, followed by the NSTEMI and UAP. When dividing the patients into subgroups based on the cutoff value for GFR (<60 ml/min), was discovered a statistically significant difference between the patients with different diagnosis. Patients with STEMI diagnosis with GFR<60ml/min are significantly more than those in the group with UAP (p=0. 006), and those with NSTEMI rarely have values of GFR<60 ml/min from UAP. These data are consistent with those reported in the literature (Hanna EB., 2011, Levin A., 2014, Tores I., 2017).

Regarding gender, we confirmed that GFR in males is significantly higher compared to females in line with other authors (Marenzi G., 2012, Sarnak MJ., 2003). Logically, glomerular filtration rate (GFR) decreases with age regardless of gender. The findings confirmed that individuals with diabetes have significantly lower GFR, which is consistent with previous studies and was expected (Hanna EB., 2011, Green SM., 2011). The result showing significantly higher values of glomerular filtration rate (GFR) in current smokers compared to ex-smokers and non-smokers was interesting. Comparable results were observed in different age groups (except for the oldest age group) as well as when grouping by gender. The significantly lower values of glomerular filtration rate (GFR) and the tendency for higher levels of serum creatinine in hypertensive patients, regardless of gender, were also confirmed in our results.

Task 3: Early risk stratification in ACS using established clinical practices contributes to faster and more effective treatment of patients. Assessment of left ventricular (LV) function through echocardiography is recommended and an integral part of cardiology practice. Furthermore, there is limited data on the factors influencing ejection fraction (EF) among the population with acute coronary syndrome (ACS) and varying degrees of left ventricular dysfunction. In the ACSIS study the authors report that patients with severe left ventricular (LV) dysfunction were older and had a significantly higher frequency of comorbidities, which is confirmed in our study regardless of gender (Brezinov O., 2017). Our results confirm the published data that the patients with atherosclerotic involvement of a single coronary vessel have more often EF>50% in comparison with patients with double- and triple-vessel (Brezinov O., 2017). In Furtado's study, the group with severe systolic dysfunction (EF <40%) had a higher frequency of acute left-sided heart failure (>II Killip),, renal insufficiency, previous myocardial infarction (OMI), and multivessel disease, which coincides with the results obtained in our study. Regarding the overall cumulative mortality based on ejection fraction, the same authors reported a 36% overall mortality in the group with EF <40%, which is similar to the results obtained in our study (44%). In 2012, Lee et al. investigated the overall survival of Korean patients with acute coronary syndrome (ACS) and left ventricular systolic dysfunction (EF<40%). They divided the patients into two groups based on their survival for less than five years or five years or more. On the 30th day after discharge, they found a cumulative survival rate of 88.0%, 78% at 6th month, 75% at 12th month, and a 5-year survival rate of 40%, which is consistent with the current results. They observed that among non-survivors, there were more females aged over 65 years with elevated levels of serum creatinine and EF<30%. Using Cox regression analysis for survival, the same author group identified that EF<30%, age over 65 years, female gender, and serum creatinine levels above 3.0 mg/ml were independent predictors of an unfavorable prognosis. Other authors as Kontilla

K.at al. have also reported significantly poorer short-term and long-term survival in females (51.0% vs. 35.9%, p=0.002). In the current study the worse prognosis for females was confirmed up to the 6th month, but on the 5th and the 9th year the survival rate was not dependent on the gender. This result may be attributed to the lack of data on causes of death in the fifth and ninth years of the study, which we acknowledge as a limitation. In terms of the studied factors at the 1st and the 5th year we confirmed that the individuals with EF<40% have worse overall survival compared to those without, regardless of gender. We discovered that triple-vessel coronary disease accounts for the largest share in NSTEMI (52%), while in UAP is 30%, and STEMI 25.7%. Logically, patients with STEMI most often have single-vessel coronary disease (39.6%). According to the demographic indicators, in line with the reported in the literature data, we did not find a significant correlation between the number of affected coronary vessels and gender. However, in terms of age, we found that patients with triple-vessel coronary disease were the oldest (Brezinov O., 2017, Furtado R., 2023). Literature data on the association between the levels of oxidative stress (OS), LDL-C, HDL-C, and triglycerides (TG) with the extent of coronary vessel involvement are limited. In our study, we also sought to establish a relationship between these parameters and the degree of coronary vessel involvement in our study group. We did not find any significant correlation between the levels of oxidative stress (OS), LDL-C, HDL-C, and triglycerides (TG) and the number of affected coronary vessels. Additionally, no significant associations were observed with other modifiable risk factors, except for renal insufficiency, specifically in individuals with moderate to severe renal impairment and reduced GFR<60 ml/min (41.5%) had a tendency for triple-vessel coronary disease (p=0.098), which is consistent with Gibson CM., 2004, Vinod V.and Yousif Z., 2021. We did not find a correlation between the number of risk factors and the number of affected coronary vessels. In other words, among patients with triple-vessel coronary disease, there was no significant increase in the prevalence of risk factors. This indicates that there is

no linear increase of the risk when the number of RF is increased, i.e., the likelihood of a patient having triple-vessel coronary disease is the same for a patient with one risk factor as it is for a patient with three or more risk factors Brezinov O., 2017, Vinod V.and Yousif Z., 2021, Furtado R., 2023.

Tasks 4 and 5. There is a tendency for higher in-hospital mortality in patients with three-vessel involvement and high GRACE (p= 0. 003). There is a statistically significant correlation between the presence of acute left-ventricular cardiac insufficiency and death during hospitalization (p=0. 017), regardless of the involved vessel (LM, LAD, LCx, RCA), as reason for ACS. The study of Aguado-Romero M. et al., the overall in-hospital mortality rate was 9.6% (4,401 cases out of 46,007), with a significant difference between genders - 11.8% for women and 8.3% for men. Adjusted for age, the mortality rate is significantly higher in advanced age and varies significantly based on the diagnosis. These results are consistent with our study, where the overall in-hospital mortality rate is 7.6%, with a significant difference between genders (p=0.037). This difference is also present in terms of the risk of mortality at the 6th month between genders (p=0.048), more women (47.5%) having higher risk for death from ACS than men (29.2%). In our study, the GRACE score is confirmed as a short-term factor determining the risk of mortality not only during hospitalization and at 6 months but also as a long-term factor in patients with ACS. The EPICOR study reports a 12% mortality rate in the first year, and Ellis et al. following up patients with ACS for an average of 12.7 years found an all-cause mortality of 52%, with the group with ST-elevation myocardial infarction at 58%, non-ST-elevation myocardial infarction at 61%, and unstable angina at 42% (p < 0.0001). In our follow-up of average 9 years, the all-cause mortality is 39.2%. From 1st to 4th year after discharge we observe almost constant survival of patients with unstable angina and parallel curves of STEMI and NSTEMI with better survival of the first group. Around the 8th year there is a crossing of the curves with worsening of the survival in individuals with experienced STEMI.

Task 6: The obtained values are close to those reported in studies for other control groups and patients with acute coronary syndrome, which also show significant differences (Lee D., 2012, Kontilla K., 2021, Furtado R., 2023). We obtained significantly lower values of the activity of PON1 in patients with acute myocardial infarction with elevation of the ST-segment and unstable angina in comparison with the control groups, which confirms the published results in the literature (Mackness, M.I., 2002, Yang-Ping L., 2002, Kumar A., 2008). The observed tendency of lower values of the enzyme activity in patients with triple- and multi-vessel coronary artery disease and hypertension confirms the results of other studies (Granér M., 2006, Wheeler JG., 2004). The Caerphilly Prospective Study investigated enzyme activity in clinically healthy men who were followed for 15 years. It was found that PON1 activity was 20% lower in those who had experienced a cardiovascular event (p = 0,039). It was interesting that we did not receive the expected proportional correlation between two enzyme activities of PON1. This observation could be explained by the presence of different genotypes of the two functional polymorphisms in the gene PON1 [Gln(Q)192Arg(R) and Leu(L)55Met(M)] and the proven differences in the genetically determined variants of enzyme for different substrates, including paraoxon and phenylacetate. Since elevated levels of LDL are considered one of the major risk factors, proper metabolism, and protection of LDL from oxidative and/or glycation modification are the focus of many studies aiming to identify new targets for therapy and early prevention. Among the factors involved in the protection of LDL from oxidative and glycation modification is the enzyme PON1, carried by HDL and contributing to the antioxidant properties of HDL by hydrolyzing lipid peroxidation products, including those in LDL (Abudayyak M., 2020, Garin M., 1997). The results from a large number of studies that have reported cross-sectional associations between the PON1 L55M polymorphism and various cardiovascular diseases yield conflicting results. In several studies, the variant allele or genotype of the PON1 L55M polymorphism are predictors of CVD, while in others, there is no

significant difference in genotype frequencies between patients and controls, and these studies fail to find an association with ischemic heart disease (IHD) and acute myocardial infarction (Kallel, A., 2010, Arca M., 2002). We evaluated the role of the polymorphism of PON L55M in acute coronary syndrome (ACS) among the Bulgarian population and discovered a statistically significant difference in the frequency of the genotype between patients and control subjects. The heterozygous genotype (LM), as well as the genotypes with variant M allele (LM+MM) seem to define over 2.5 times higher risk for developing ACS, which is not dependent on factors such age and gender. More specifically, the genotype PON1 55MG and the genotypes with variant M allele are predisposing factors for STEMI, while for UA or NSTEMI these associations are not significant. Our results are consistent with those of Bounafaa et al., reported in 2015, which also demonstrate that the PON1 55MM genotype is associated with a higher risk of ACS compared to individuals with the LL genotype (OR=3.69; 95% CI=1.61–11.80). When we compared different biochemical markers between genotypes L55M PON1, we identified correlations only in the group of patients with NSTEACS: these patients with genotypes with at least one variant of M allele (LM+MM) have significantly higher serum levels of total cholesterol (TC) and triacyl glycerides (TAG), than the patients with LL genotype. However, these associations have not been observed in patients with STEMI, as well as in the entire group of patients with ACS. The literature contains limited and conflicting data regarding the role of PON1 L55M genotypes in patients with ACS, as well as their potential impact on the survival of this group of individuals (Sökmen et al., 2019). Due to the lack of literature data on the association between the calculated GRACE score at hospitalization and PON1 L55M SNP genotypes, we analyzed it, but such an association was not observed in our results. There was also no connection found between short-term mortality risk (in-hospital mortality, GRACE result 108/109-139/≥140) with the genotypes of PON1 L55M SNP (p=0.436). Our results did not show an association between the genotypes of

PON1 L55M SNP with the in-hospital and 6-month survival of the individuals. No significant associations were found between the distribution of genotypes and long-term mortality risk (GRACE score 88/89-117/≥118, p=0.302). No association was found between 1-year survival and PON1 L55M genotypes, nor in the entire group of patients (p=0.640), nor in the subgroups with different diagnosis (p=0.902 for STEMI; p=0.108 for UA+NSTEMI). However, it is worth noting that in the NSTEACS subgroup, all patients (n=13) with variant M allele genotypes (LM+MM) were alive at the end of the first year, while 2 of the patients with the LL genotype (18.2%) were deceased. After 5 years of follow-up the alive patients are in total 49 (63,6% survival rate), and again no associations with PON1 SNP (p=0.561) have been observed. There is currently a lack of published data on the impact of PON1 SNP L55M on the nine-year survival rate after ACS (Acute Coronary Syndrome). We searched for information on this matter but did not find any association with PON1 SNP3 (p=0.389), and the percentage of survival is 50.6% (39 of 77). In a Chinese study, the relationship between the presence of PON1 SNP L55M was investigated in patients with ischemic heart disease (IHD), revealing a weak correlation (195). However, we did not find a similar association in our patient group. Sökmen et al. analyzed the association between the calculated SINTAX score, the localization of myocardial infarction (MI), and the presence of PON1 SNP L55M in patients with ST-elevation myocardial infarction (STEMI), but the authors did not find a correlation. In this regard, we searched for an association with the short-term mortality risk (in-hospital mortality, GRACE score 108/109-139/≥140) with the genotypes of PON1 L55M SNP (p=0.436). No significant associations were found between the distribution of genotypes and the long-term risk of mortality from all causes (GRACE score 88/89-117/≥118, p=0.302). The Kaplan-Meier survival curve after one year of follow-up for the patients shows that, although not significant, patients with the LL genotype have a favorable prognosis (average survival period of 17.81 months) compared to patients with M allele genotypes (LM+MM), with an

average survival period of 20.31 months (p=0.553, Log rank test). This difference was more pronounced in patients with NSTEACS (p=0.115). However, over the course of a five-year follow-up (60 months), the apparent difference in survival between patients with LL and those with M allele genotypes (LM+MM) completely disappears. Even if the long period of 9-year (108-months) follow-up, although insignificantly, the prognosis of the patients with LL genotype becomes more favorable than the one of the patients with LM or MM genotypes, especially for patients with NSTEACS.

Task 7. During the univariate analysis, we found that significant adverse prognostic factors for survival up to 1 year include diagnosis (STEMI), age, and GFR, GRACE-score >140 p., presence of ALSHF>II Killip at hospitalization, cardiogenic shock (p=0.013), AV block (p=0.039) and TVD (p=0.027), experienced AMI and ejection fraction of LV (EF<40%), which is confirmed in a series of studies (Alhabib KF., 2012, Brezinov O., 2017, Pocock S., 2015). For the 5th and 9th year period the same variables are identified significant independent prognostic factors. Additionally, two more factors emerged as significant predictors of survival: tobacco smoking and diabetes mellitus. Significant and independent predictors for mortality from the multivariate Cox analysis for the 5-year and 9-year survival periods include cardiogenic shock (p=0.013), AV блок (p=0.039), TVD (p=0.027) and GRACE – score >140 p.

6. CONCLUSIONS

- The age of onset of ACS is significantly more advanced in women compared to men;

- Patients with STEMI are significantly younger than those with NSTEMI.

- Risk factors such as hypertension and smoking are significantly more prevalent among males.

- Diabetes mellitus is significantly more prevalent among individuals in advanced age.

- Young men (<49 years) have significantly lower HDL levels.

- There is a confirmed positive correlation between BMI and triglycerides (TG), and a negative correlation between HDL and BMI in both genders.

- Patients with STEMI and a GFR <60 ml/min are significantly more common than those in the non-ST-elevation ACS group.

- GFR is significantly higher in men compared to women, and as age advances, GFR significantly decreases while serum creatinine levels increase. Smokers have significantly higher GFR values.

- Individuals with diabetes mellitus have significantly lower GFR values regardless of gender.

- Patients with ACS and occlusion of the left anterior descending artery (LAD) have a significantly higher prevalence of EF <49% compared to those without occlusion of this artery.

- Triple-vessel coronary disease is most prevalent in non-ST-elevation ACS (NSTEMI) cases (52%), followed by unstable angina (UA) cases (30%), while it is 25.7% in STEMI cases. Patients with STEMI most commonly have single-vessel coronary disease (39.6%).

- A significantly smaller percentage of patients with EF <30% survived after the first year (44.4%) compared to those with higher ejection fractions.

- Up to the 5th and 9th year, survival significantly depended on the ejection fraction at hospital admission.

- The calculated individual risk score (GRACE score) is a significant predictor of mortality both during hospitalization and at the 1st, 5th, and 9th-year follow-ups. Lower GFR values and a tendency towards higher

serum creatinine levels were observed in patients with arterial hypertension. Regarding the association between GFR and gender, the differences are also statistically significant.

- The mean values of paraoxonase and arylesterase activity differed significantly between patients and control individuals, with significantly lower values observed in patients with both STEMI and UA.

- Serum paraoxonase and arylesterase activity values were significantly lower in patients with triple-vessel coronary disease, diabetes mellitus, hypertension, and those who experienced acute myocardial infarction.

- The distribution of the L55M PON1 genotype determines more than a two-fold higher risk of developing acute coronary syndrome when the individual carries genotypes with the variant M.

- Cox regression analysis showed significantly higher survival rates among patients diagnosed with unstable angina (UA) compared to those with STEMI at the 9th-year follow-up (p=0.028), and no significant difference was found between STEMI and NSTEMI.

- Kaplan-Meier curves at the 9th-year follow-up intersect for NSTEMI and STEMI, indicating a decline in overall survival in the latter group, while the curve for UA shows minor fluctuations.

- Unfavorable prognostic factors for survival up to 1 year include the diagnosis of ST-elevation myocardial infarction (STEMI), age ≥60 years, glomerular filtration rate (GFR) <60 ml/min/m2, GRACE score >140, presence of Killip class >II at hospitalization, presence of cardiogenic shock (p=0.013), AV block (p=0.039), total vessel disease (TVD) (p=0.027), previous acute myocardial infarction, and left ventricular ejection fraction (LVEF) <40%.

- Independent predictors for mortality from the multivariate Cox analysis for the 5-year and 9-year survival periods are only cardiogenic shock

(p=0.013), AV block (p=0.039), TVD (p=0.027), and GRACE score >140 p., indicating significant independent prognostic factors.

7. CONTRIBUTIONS

Original

1. For the first time, serum paraoxonase and arylesterase activity, as well as the presence of PON1 SNP L55M, are investigated among the Bulgarian population, including both healthy individuals and those with ACS. The obtained results provide a basis to suggest its possible role in the pathogenesis of atherosclerosis, particularly ACS.

2. The influence of PON1 SNP L55M carrier status on the short-term and long-term survival of patients with ACS (1st, 5th, 9th years) is investigated for the first time, but no significant association is demonstrated.

3. Prognostic models for survival and mortality due to all causes in ACS patients are developed, which could be useful in clinical practice for initial risk stratification of ACS patients, predicting survival (short-term and long-term), and assessing the risk of death.

4. The quantitative dependence of PON1 activity on genotype type and its significance in the short-term and long-term prognosis of this heterogeneous group of patients (1st, 5th, 9th years) is analyzed for the first time, but no association is found.

5. A prognostic model for 1st, 5th, and 9th-year survival and mortality due to all causes in ACS patients is developed, which could be used to determine survival (short-term and long-term) based on initial risk stratification at hospital admission

Confirmative

1. The well-known assertions regarding gender differences in the age of onset of ACS are confirmed.

2. Detailed stratification of patients based on the constellation of risk factors is performed.

3. The role of the GRACE score in predicting the risk of adverse outcomes during hospitalization and at 6 months is confirmed.

4. The role of conventional risk factors and their impact on short-term and long-term survival of ACS patients is confirmed.

5. Prognostic factors for adverse outcomes following ACS at 1st and 5th year are confirmed: diagnosis (STEMI), age ≥60 years, GFR <60ml/min/m2, GRACE score >140, presence of Killip class >II at hospital admission, cardiogenic shock (p=0.013), AV block (p=0.039), TVD (p=0.027), prior acute myocardial infarction, and left ventricular ejection fraction (EF) <40%.

6. Known significant predictors for poor prognosis are confirmed for 5th and 9th-year survival, including cardiogenic shock (p=0.013), AV block (p=0.039), TVD (p=0.027), and GRACE score >140.

Articles related to the dissertation:

1. Doneva-Basheva K.[1,2], Petrov D.[1], Vlaykova T. [3,4.] and Tisheva S. PREDICTORS FOR LONG-TERM PROGNOSIS AFTER ACUTE CORONARY SYNDROME. J Biomed Clin Res Volume 14 No 1, pp 31-46, 2021.

2. Doneva-Basheva K.1,3, Anastasov A., Postadzhyan A., Kamenova Z., Vlaykova T. SERUM PARAOXONASE AND ARYLESTERASE ACTIVITY OF PON1 IN ACUTE CORONARY SYNDROME. Trakia Journal of Sciences, No 1, pp 39-49, 2013.

Article related to the dissertation IF-factor:

1. Doneva-Basheva K.[1,2], Gospodinov K. [1], Tacheva T.[3], Dimov D.[3] and Vlaykova T. [3, 4]. Role of single nucleotide polymorphism L55M in the *Paraoxonase 1* gene as a risk and prognostic factor in acute coronary syndrome. Curr. Issues Mol. Biol. 2022, 44, 5915–5932. https://doi.org/10.3390/cimb44120403. (IF=2.976);

Participation in international forums:

1.Yordanova K., S. Emin, K. Doneva, V. Tsoneva, D. Dimov, Z. Kamenova, T. Vlaykova. Evaluation of paraoxonase and aryl esterase activity of serum PON1 in patients with acute heart disease. European Journal of Medical Research. Volume 14/ Supplement II, October 4, 2009 (Abstract Book, ESC- ID 856, 122-123). (IF= 1,04; 2009);

2. Z. Kamenova, Vl. Zhelev, K. Doneva-Basheva, Zl. Arnaudova, Zh. Andreev, S. Valcheva, M. Sindzhirlieva, V. Tsoneva. Short-term prognosis in acute coronary syndrome. (poster), VIII Scientific conference "Hearth-lungs", May 29-30, 2009, Varna, Schedule page 6.