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Effect of trimetazidine on ischemia reperfusion injury in on pump coronary surgery

Author's abstract
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Abbreviations used:

AH - arterial hypertension
ECG - electrocardiogram
ECC - extracorporeal circulation
MDA - malondialdehyde
CHD - coronary heart disease
SBP - systolic blood pressure
WHO - World Health Organization
FI - ejection fraction
COPD - chronic obstructive pulmonary disease
ALAT - alanine aminotransferase
ANOVA - Analysis Of Variance
ASA - acetylsalicylic acid
ASAT - aspartate aminotransferase
CPK - creatine phosphokinase
CPK-MB/CK-MB - creatine phosphokinase - fraction MB
CVVHDF- continuous veno-venous haemodiafiltration

ELISA - enzyme-linked immunosorbent assay
NYHA - New York Heart Association
MDA - malondialdehyde
TMZ - trimetazidine
TrT - troponin T

I.Introduction

The concept that oxygen reperfusion in the ischemic myocardium causes significant damage has long been known [1]. It is also well established that oxidative stress is one of the major initiators of myocardial injury during ischemia and reperfusion [2]. Coronary surgery usually involves cardiopulmonary bypass with cardioplegic arrest and induced global cardiac ischemia. Viewed from a research perspective, this can be considered as a model for assessing the potential damage induced by oxidative stress during ischemia and subsequent reperfusion, since in this clinical situation (and unusually for most ischemic conditions) the time of onset and duration of ischemia as well as reperfusion are controlled.

This means that studies are possible in patients undergoing surgery with a design similar to that of experimental studies involving animal models. In experimental studies on rat hearts, ischemia and reperfusion generate oxygen-derived free radicals; the use of electron spin resonance techniques allows the direct detection of reactive oxygen species formed during reperfusion of ischemic myocardium [3] and also indicates that their production can be prevented by various antioxidants or neutralizing agents [4].

A large number of experimental studies that simulate surgical ischemia and reperfusion have shown that various antioxidant agents can reduce the deleterious effects of oxidative stress resulting from ischemia and reperfusion and lead to improved cardiac function after ischemia, when the agents are added pre- or during plexus as a constituent of the cardioplegic solution [5,6].

Cardiac surgery also induces "stunning" or stunting of the myocardium (seen as transient cardiac dysfunction) in the first few hours after reperfusion [7,8], and it might be expected that this should also improve with the administration of antioxidants. So one might speculate that oxidative stress induced during cardiac surgery may be a factor to focus on in our quest to improve postischemic recovery. At the same time, there is a substantial difference between studies in animal models, usually with healthy hearts, and clinical studies of patients with ischemic disease requiring cardiac surgery, whose myocardium is much more tolerant of injury because of activation of endogenous adaptation mechanisms, such as ischemic preconditioning. The administration of a drug such as trimetazidine, whose action on oxidative stress has a similar effect, may have an important role in recovery after cardiac surgery. The question of whether oxidative stress influences postoperative outcomes in patients after coronary surgery has not yet been definitively answered. Patient patterns are difficult to unify and the influence of many factors that may affect outcomes, such as comorbidities, complications that may arise, differences in preoperative and postoperative therapy, cannot be ignored.

The interpretation of results and conclusions drawn in these conditions can be misleading. The aim of our study was to select patients in the groups with as similar baseline characteristics as possible, undergoing the same surgical procedure and drug therapy, to largely avoid these negatives. We looked for an effect of trimetazidine in the study group entirely postoperatively, because in real practice coronary surgery is often in urgent or emergency order and without conditions for prolonged preoperative drug therapy, as in previous studies by other authors.

II. Aim and objectives

1. Objective:

   To investigate the effectiveness of postoperative administration of trimetazidine on the reduction of oxidative stress and postoperative damage during the ischemia-reperfusion period.

2. Objectives:

a. Determine baseline characteristics of the study population and define groups;
b. Analysis of intraoperative data and postoperative outcomes and comparison between groups;
c. Six-month follow-up of the patients and monitoring of the determined parameters: FI, MB-SC levels, TgT and MDA;
d. Evaluating the efficacy of postoperative trimetazidine therapy on oxidative stress and myocardial functional capacity;
e. Assessment of the real benefits of postoperative trimetazidine administration.

III. Material and methods

1. Type of study

The study was a prospective, blinded, randomized, controlled trial.

2. Object of observation

The study population were 93 patients who underwent elective isolated coronary surgery in the Department of Cardiac Surgery at St. George Hospital - Plovdiv between March 2018 and October 2018. Patient inclusion was prospective by performing pre-specified inclusion and exclusion criteria. Voluntary participation of all subjects was declared by written informed consent.

All patients underwent cardiac surgery under ECC with aortic valve clamping and cardioplegic arrest.

Of the total number of patients, two developed acute renal failure requiring continuous veno-venous haemodiafiltration (CVVHDF) and one patient required prolonged mechanical ventilation (more than 48 hours) and were therefore excluded from this study.

The final study group included 90 patients previously divided into 2 subgroups: a first group (n=45) receiving trimetazidine 35 mg twice daily immediately after extubation; and a second control group (n=45) receiving placebo. Remaining therapy was identical for all participants: acetylsalicylic acid (ACA) 100 mg/day, rosuvastatin 10 mg/day, metoprolol 50 mg/twice daily, perindopril 5 mg/day and gliclazide 60 mg/day.

The mean age of patients for the study group was 62.97 ± 9.45 years. Of these, 11 or 24.4% were women and 34 (75.6%)- men, the male/female ratio

was 3/1. The mean age for the control group was 64.18 ± 6.54 , the male/female ratio was the same.

3. Units of observation

3.1. Logical units

The study population consisted of 90 patients who underwent elective isolated coronary surgery in the Department of Cardiac Surgery at St. George Hospital – Plovdiv.

The number of survey units required to ensure 95% confidence in the scientific information was calculated using the Stein method.

3.2. Technical unit - Study site. St. George Hospital - EAD, Medical University - Plovdiv, Department of Cardiac Surgery.

4. Study period

The survey was conducted between the months of March 2018 and April 2019. The required observation units were registered during this period.

5. Conducting the survey

The study used one of the basic sociological methods - documentation. Registration and postoperative follow-up protocols were used (Appendix 1), as well as measurement of human malondialdehyde (MDA) by ELISA (Appendix 2).

6. Parameters studied

The data obtained in this prospective monocentric study were extracted from the available records of the Cardiac Surgery Unit between the months of March 2018 and April 2019, as well as the medical history of the patients during the study period.

6.1. Signs of observation

The two basic groups of signs are examined.

Main factorial signs:

- **Age**

  At the time of admission to the Department of Cardiac Surgery. A continuous variable was used to analyze the age parameter.
- **Gender**

  A binary variable was used, coded as a nominal variable for statistical analysis: 1 - men, 2 - women.
- **Systolic blood pressure (SBP) and pulse as the main vital signs**

  A continuous variable was used for the statistical analysis.
- **Laboratory tests including**

  Complete blood count and biochemistry with measurement of glucose, urea, creatinine, uric acid, WBC, WBC-MB, TgT, ASAT, ALT, bilirubin, fat profile, electrolytes and blood gas analysis performed at admission and the evening before surgery.

- **Electrocardiography**

ECG was performed in all patients. Baseline on admission and daily in the early postoperative in-hospital period. Follow-up during follow-up examinations as per accepted protocol: ECG recording was in 12 leads, at a paper speed of 25 mm/s and a gain of 10 mm/mV.

- **Echocardiography**

It was performed according to protocol in all patients during the initial examination on admission, as well as during the stay until discharge and at follow-up examinations during follow-up. The examination was performed transthoracically. Ejection fraction was examined by the Simpson method. Baseline FI was noted in the registration protocol. Patients with an FI below 40% were excluded, as were those with valvular pathology requiring surgical correction. Follow-up values are noted in the postoperative follow-up protocol.

- **Heart and lung radiography**

Examination was performed in all patients preoperatively, on the first postoperative day, and at their discretion until discharge. Inflammatory changes of lung parenchyma, areas of atelectasis, cardiothoracic index, presence of pleural and pericardial effusions were looked for.

- **Cardiac catheterization and coronary angiography**

All patients in the study had undergone angiography with evidence of triclonus coronary disease indicated for elective surgical treatment.

- Operative intervention

All patients were operated in the Cardiac Surgery Department of St. George Hospital - Plovdiv. The type of surgical approach used was median longitudinal sternotomy. All cardiac surgeries were performed under the conditions of ECC and aortic valve clamping with cardioplegic arrest secured, by infusion of cold blood cardioplegia retrograde into the coronary sinus and antegrade into the aortic root, according to the protocol of the department. The operations were performed under moderate hypothermia. In all patients, the left mammary artery was dissected and used as an arterial graft to the left anterior descending coronary artery. The remaining target coronary arteries were revascularized using saphenous vein grafts.

6.2. Main resultative signs:

Patients were followed up to the 6th month. Results were recorded at the 1st and 12th hour after surgery, at discharge, and at the 1st and 6th month after surgery.

All patients received identical drug therapy postoperatively, except TMZ.

The follow-up was conducted as follows:

- the 1st hour after surgery:
    - ejection fraction

- on the 12th hour after surgery:
    - serum levels of CK-MB
    - serum levels of TrT
    - serum levels of MDA

- at discharge:
    - ejection fraction

- the 1st month after surgery:
    - serum levels of MDA

- at the 6th month after surgery:
    - ejection fraction
    - serum levels of CK-MB
    - serum levels of TrT
    - serum levels of MDA

The comparison of the results of the patients of the main groups was performed according to the chosen statistical methods according to the type of characteristic (quantitative or qualitative) and its distribution.

Subgroups were formed based on the ranges of interest of certain indicators, with sub-selections made within the main groups. A comparison of baseline characteristics was made to demonstrate comparability between samples.

7. Sources of information

- Patients' medical history
- Registration protocols including: preoperative data (FI, CPK-MB, TgT, MDA, diabetes mellitus and AH), surgical intervention (ECC, clamp, number of bypasses, venous grafts).
- The postoperative follow-up protocol including: total ICU stay (in hours), total hospital stay (days), total blood loss(in ml).

  FI at the 1st hour, at dehospitalization, at the 6th month.

  Serum CK-MB levels: at 12th hour, 6th month.

  Serum TgT levels: at 12th hour, 6th month.

  Serum MDA levels: at 12th hour, at 1st month, at 6th month.

  Measurement of human malondialdehyde is by ELISA.

8. Research methods

8.1. Clinical methods

8.1.1. History and physical status

Anamnestic data were collected using a study-specific registration and postoperative follow-up protocol including age at admission, sex, preoperative data, surgical intervention, and postoperative follow-up (Appendix 1).

The history establishes the main symptoms of CHD, their severity and nature, and also establishes the NYHA functional class of each patient, the presence of familial, harmful habits and concomitant diseases. Anamnestic data are also sought from accompanying medical records for the presence of exclusion criteria.

A complete physical examination was performed according to the generally accepted rules of cardiac examination and was performed in all patients included in the study. The degree of clarity of consciousness, habitus, bed position, skin colour and visible mucous membranes, jugular venous stasis and peripheral lymph nodes were noted.

The examination of the lung includes: percussion and auscultation, the type of breathing, the duration of inspiration and expiration, the presence of a wheezing finding.

The status of the heart takes into account: rhythm, frequency, auscultatory assessment of heart tones, the presence of pathological tones and noises and their characteristics, pericardial friction.

The abdominal status reports the location relative to the chest, tenderness on palpation, symptoms of peritoneal irritation, auscultatory characteristic of peristalsis, and liver dimensions determined by palpation and percussion. A succussion renalis was performed.

The limbs were examined for skin color and temperature, spontaneous palpatory and movement-related tenderness, venous engorgement, presence of edema, their location and symmetry, peripheral pulse was palpated at the access sites for all four limbs - presence or absence, rhythmicity, engorgement, symmetry. Peripheral arterial pressure was measured.

8.1.2. Clinical-laboratory methods

All patients were examined according to the protocol of the Department of Cardiac Surgery at St. Blood counts and biochemistry with glucose, urea, creatinine, uric acid, WBC, WBC-MB, TgT, ASAT, ALAT, bilirubin, fat profile, electrolytes and blood gas analysis were performed at admission and the evening before the surgery.

Patients showing abnormalities in renal function or with evidence of COPD were excluded from the groups. MDA values were measured 12 hours before surgery.

8.1.3. Instrumental methods

Instrumental examinations include: electrocardiography, echocardiography, radiography of the heart and lungs, as well as cardiac catheterization and coronary angiography.

Diagnosis and selection of the patients was performed according to the inclusion and exclusion criteria set in an individual "Protocol for registration and follow-up", specially designed for each patient. All the steps and the recording of the results were reflected in them. In addition, the human malondialdehyde (MDA) measurement system was used by ELISA method.

9. Statistical methods

The collected statistical information was processed in the Department of Medical Informatics, Biostatistics and E-learning at the Faculty of Public Health, Medical University - Plovdiv.

The data recorded in the individual protocols were entered into Microsoft Excel 2010 and IBM SPSS Statistics v. 17 spreadsheets.

Starting from the main aim and objectives of the study, as well as the volume, type and distribution of the data, the following statistical methods were used:

1. Descriptive statistical methods:

    - Quantitative metrics are presented with baseline measures of central tendency, statistical dispersion, and confidence interval (mean ± SD, Se, 95% CI);
    - Qualitative, unmeasured quantities are represented by absolute frequencies, relative proportions, and standard error (counts, %, Sp)

2. Parametric and non-parametric methods

Fisher's exact test for fourfold tables and the x^2 criterion for multiple tables were used to test the hypotheses of non-significant (random) influence of a factor.

- The Kolmogorov-Smirnov test was used to examine the normality of the distribution of the studied indices.

- To test hypotheses for the presence of statistically significant difference between two independent samples with examined quantitative, normally distributed indicators, Student's t-test was used, when comparing quantitative variables in independent samples with a distribution other than normal - Mann-Whitney test.
- Student's independent-samples t-test was used to test hypotheses about the presence of a statistically significant difference between two dependent samples by examining quantitative, normally distributed indicators. When comparing quantitative variables in independent samples with a distribution other than normal, the Mann-Whitney U-test was used.
- For comparison of quantifiable normally distributed indices in more than 2 groups, analysis of variance (one-way ANOVA). For comparison of more than 2 independent samples with data distribution different from normal - Kruskal-Wallis test.
- The relationship between the studied indicators was analyzed using correlation analysis. Pearson's coefficient is presented for normally distributed variables. For variables with different from normal distribution Spearman correlation coefficient is presented.

The significance level of the null hypothesis was taken as $p<0.05$. Statistical data processing was performed using the SPSS Statistics v. 17 software.

3. Graphical methods - Microsoft Excel 2010 was used for graphical presentation of the results.

IV. Results

During cardiac surgery with cardiopulmonary bypass and aortic valve replacement, a series of biochemical and immunological changes lead to an increase in oxidative stress of the cardiomyocyte. Under the non-physiological conditions of extracorporeal circulation, an increase in free radical levels and reactive oxygen species occurs, leading to cell damage.

Additionally, reperfusion of the myocardium previously ischemic during aortic valve replacement also results in significant damage. This ischemia-reperfusion stress is the cause of the deepening and extension of myocardial damage.

It is assumed that the reduction of oxidative stress will reflect on biochemical and vital parameters of the myocardium, i.e. we should report a decrease in CPK-MB and TrT levels and an increase in FI. Data from previous studies are inconclusive. Some of them show an increase in FI [107][109] while others do not record such [102][108], most give evidence of a decrease in cardiac enzyme levels [103][109][112].

One goal of the study is to find out if we can affect oxidative stress by administering a medication that is easily administered, widely available, financially unburdensome, and with minimal risk for side effects. Our other goal is, by monitoring functional parameters and biomarkers, to see how much reduction in oxidative stress, if any, leads to a reduction in myocardial damage and hence for practical application, whether inclusion of trimetazidine to the therapy protocol for patients after bypass surgery is warranted.

A cohort of patients with as similar baseline characteristics as possible undergoing the same surgical procedure was selected for this purpose. The patients were divided into two groups of 45 each, a treatment and a control group of 90 patients in total. Of the 90 patients, 67 (74.44%) were male and 23 (25.56%) were female.

In addition to demographics, factors related to the surgical intervention that may lead to differences in oxidative stress levels in the two groups before the start of trimetazidine treatment are also a consideration. To this end, we recorded the duration of extracorporeal circulation, aortic valve clamping, and the amount of total blood loss in the two groups and compared them. The selected parameters are well traceable and objectively documented.

The sex ratio was 3:1 in favour of men, which is understandable given the higher incidence of atherosclerotic disease in the male sex.

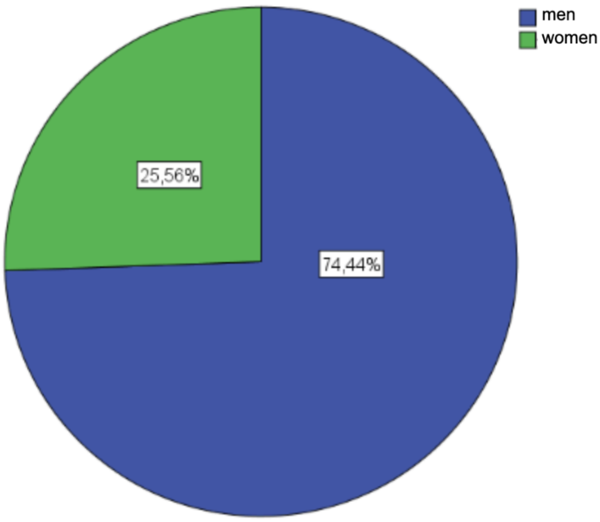


Diagram 1: Distribution of studied patients by sex.

The sex distribution in the two groups was as follows 33 to 12 for the control group and 34 to 11 for the trimetazidine-treated group. There was no statistically significant difference in sex distribution between the control group and the TMZ group (Table 1).

Table 1. Bivariate distribution by sex and study groups.

			Groups		
			Control	TMZ	Total
Gender	men	Number	33	34	67
		half	49,3%	50,7%	100,0%
		% in group	73,3%	75,6%	74,4%
	women	Number	12	11	23
		half	52,2%	47,8%	100,0%
		% in group	26,7%	24,4%	25,6%
Total		Number	45	45	90
		half	50,0%	50,0%	100,0%
		% in group	100,0%	100,0%	100,0%
		Fisher's exact test, P=1.000			

49.3% of the men were in the control group, while 50.7% were in the treatment group. Correspondingly, of the females, the percentage distribution in the two groups was 52.2% for the control group to 47.8% for the treatment group.

The sex ratio in both study groups remained 3:1 (Figure 2).

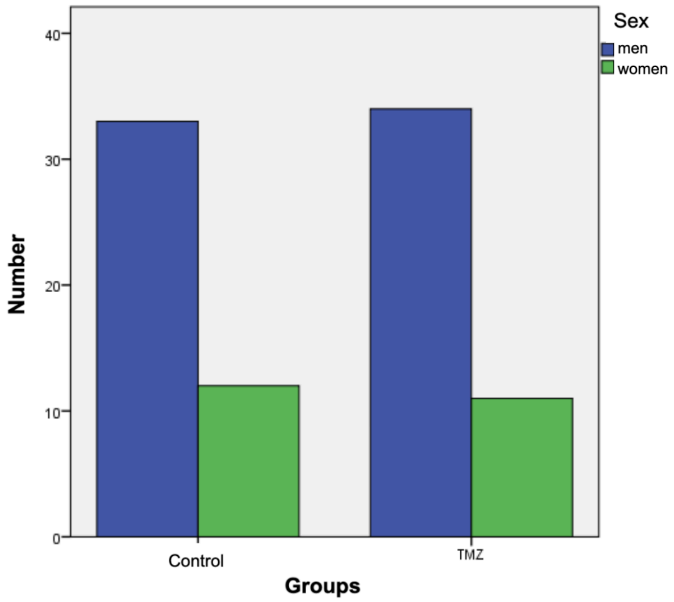


Chart 2: Frequency distribution by sex and groups

Distribution by age

Analysis of the age distribution shows that the 61-70 age group is the most affected. More than half of the patients are in this group.

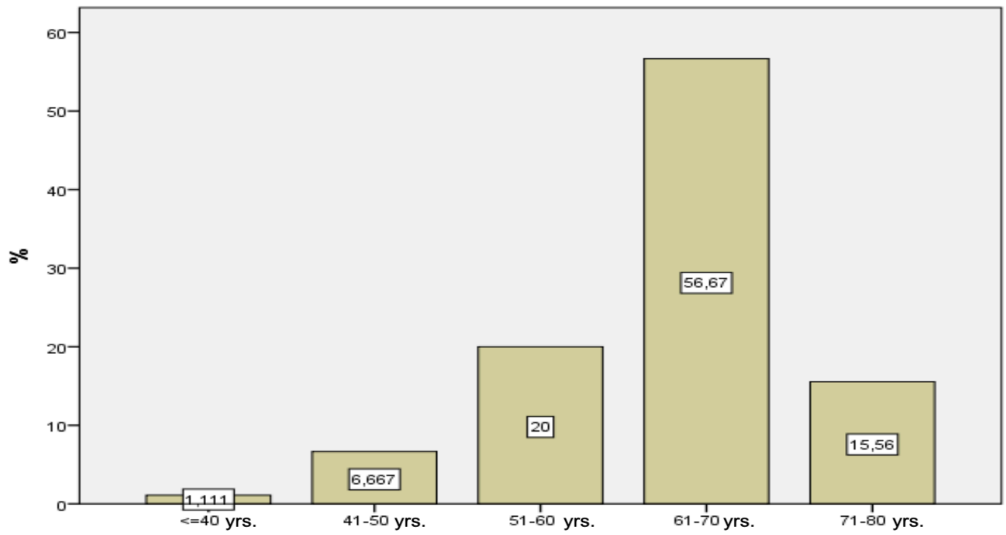


Diagram 3: Distribution of studied patients by age groups.

When the bivariate distribution by sex and age groups was examined, no statistically significant difference was found (Table 2)

Table 2. Bivariate distribution of studied patients by sex and age groups.

			Gender		Total
			men	women	
Age gr.	<=40 г.	Number	1	0	1
		% in age groups	100,0%	,0%	100,0%
		half	1,5%	,0%	1,1%
	41-50 г.	Number	4	2	6
		% in age groups	66,7%	33,3%	100,0%
		half	6,0%	8,7%	6,7%
	51-60 г.	Number	12	6	18
		% in age groups	66,7%	33,3%	100,0%
		half	17,9%	26,1%	20,0%
	61-70 г.	Number	41	10	51
		% in age groups	80,4%	19,6%	100,0%
		half	61,2%	43,5%	56,7%

	71-80 г.	Number	9	5	14
		% in age groups	64,3%	35,7%	100,0%
		half	13,4%	21,7%	15,6%
Total		Number	67	23	90
		% in age groups	74,4%	25,6%	100,0%
		half	100,0%	100,0%	100,0%
		X^2=2.81,df=4, P=0.589			

In both sexes, the 61-70 age group is the most affected.

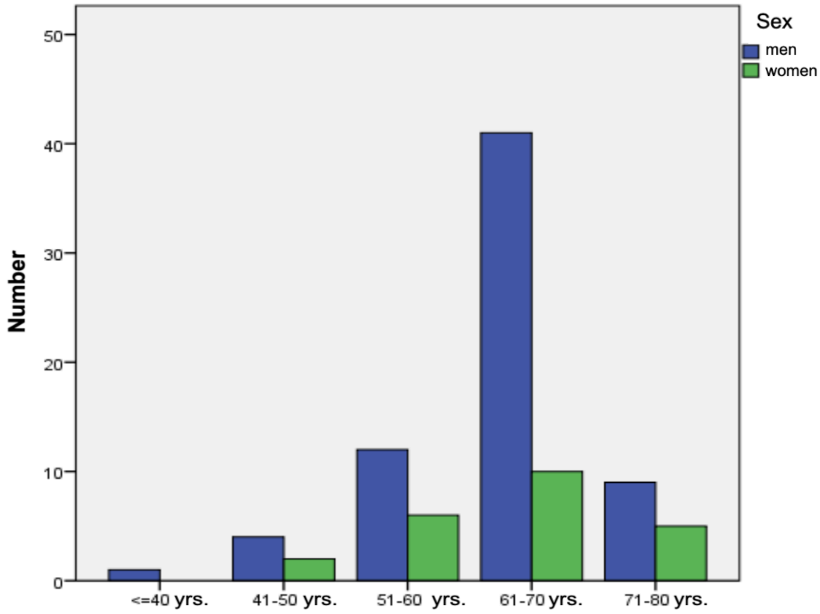


Diagram 4: Distribution of the studied patients by sex and age groups

When comparing the mean age of patients in the control and trimetazidine groups, no statistically significant difference was found

Table 3. Comparison of the mean age between the two study groups.

	Groups	N	Mean	Std. Deviation	Std. Error Mean	t	P
Age	Cotrol	45	64,1778	6,54109	,97509	0.70	0.486
	TMZ	45	62,9778	9,44960	1,40866		

This result was confirmed by examining the bivariate frequency distributions by age and patient groups examined (Figure 5).

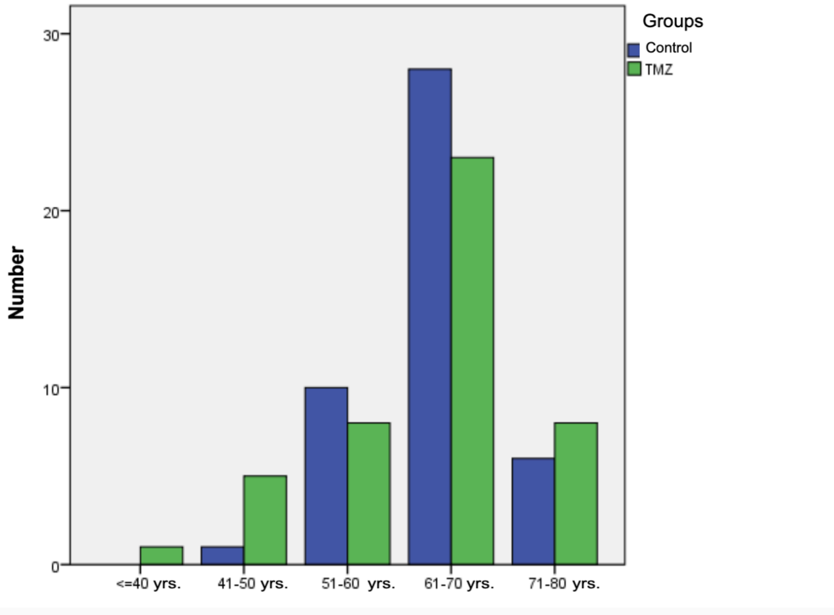


Chart 5: Frequency distribution of study patients by age group for the control and TMZ group.

The distribution of patients by age groups in the control and treatment groups showed no significant differences. In both groups, most patients were in the 61-70 years age group.

We investigated the following parameters affecting oxidative stress associated with surgical intervention.

- aortic valve duration (in minutes)
- duration of ECC (extracorporeal circulation) in minutes
- total blood loss (in millilitres)

There was no statistically significant difference between the control and TMZ groups for all three indicators examined (Table 4)

Table 4. Comparison of valve time (aortic valve duration), ECC duration and total blood loss between control group and TMZ group.

	Groups	N	Mean	Std. Dev.	Std. Error Mean	t	P
Clamping time (min.)	Control	45	28,22	2,78	0,41	1.43	0.156
	TMZ	45	27,42	2,50	0,37		
ECC duration (min.)	Control	45	43,53	3,89	0,58	0.231	0.818
	TMZ	45	43,71	3,37	0,50		
Total blood loss (ml)	Control	45	276,00	66,24	9,87	1.51	0.135
	TMZ	45	255,77	60,69	9,04		

The studies thus performed show that the two groups have the same baseline characteristics, i.e., they are comparable and can be examined for the presence of an effect or lack of an effect with trimetazidine administration.

Formation of subgroups by age

For the limit of interest, set the value 68 yrs. According to a definition adopted by the WHO in 2012 from an article published in the International Journal of Epidemiology, people over 68 years of age are considered to be adults [116].

Tracked indicators

The investigated parameters were divided into laboratory biomarkers - malondialdehyde (MDA), isoenzyme MB of creatine phosphokinase (CPK-MB) and troponin-T levels and functional ones - echographically measured left ventricular ejection fraction (EF).

3. Analysis of laboratory indicators

Laboratory markers CPK-MB and TrT

Both laboratory markers are routinely used and easily followed in cardiac surgery patients. Tracking them in both groups of patients assesses, on the one hand, myocardial damage during the specific surgical procedure and the correlation or lack thereof with the quantitatively recorded oxidative stress by examining malondialdehyde levels.

Both biomarkers increase in cardiomyocyte ischemia and necrosis, with **TrT** being more specific and rising earlier compared to **CPK-MB,** in **contrast CPK-MB** levels decline later and can be tracked longer in time. **TrT** is more indicative of ischemia and without significant damage and cell death.

3.1. Isoenzyme MB (U/l)

Creatine phosphokinase (CPK) is an enzyme expressed by various tissues. It catalyzes the conversion of creatine and uses adenosine triphosphate(ATP) to synthesize phosphocreatine(PC) and adenosine diphosphate(ATP). The reaction is reversible and ATP can be generated from ADP and FC. In cells that rapidly consume ATP, especially in skeletal muscle, brain, and myocardium, FC serves as an energy reservoir for the rapid buffering and regeneration of ATP as well as for intracellular transport via the phosphocreatine cycle.

The CF isoenzyme of CPK is used as a specific indicator of myocardial injury in the postoperative period in cardiac surgery. Its structure is that of a dimer. The subunits of the SRK-dimer are divided into M(muscle type) and B(brain type). MM-dimer is found mainly in skeletal muscle, BB-dimer in brain tissue and MB-hybrid in cardiac muscle.

Using the Kolmogorov-Smirnov test, we analyzed the distribution of the isoenzyme MV studied 12 hours before surgery; 12 hours after surgery; 6th month after surgery (Table 5). We found a different than normal distribution for isoenzyme MB at all time points of study:

Table 5. Distribution of isoenzyme MB at different time points studied (results from Kolmogorov-Smirnov test)

One-Sample Kolmogorov-Smirnov Test

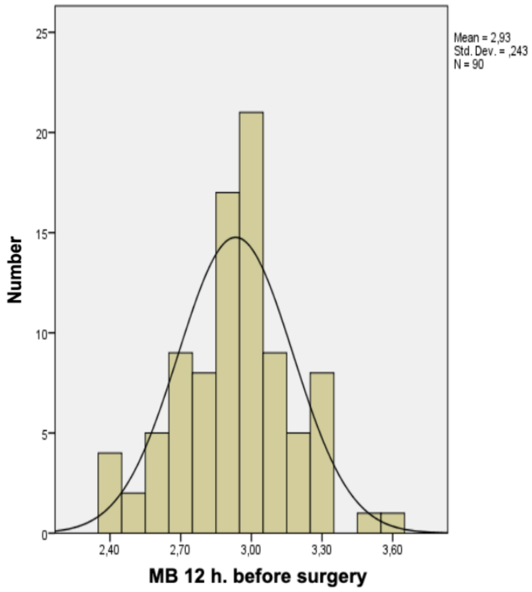
		MB 12hr preOP	MB 12hr postOP	MB 6 months postOP
N		90	90	90
Normal Parameters[a,b]	Mean	2,9333	37,4756	3,1811
	Std. Deviation	,24311	5,81973	,36747
Most Extreme Differences	Absolute	,134	,146	,087
	Positive	,125	,146	,057
	Negative	-,134	-,095	-,087
Test Statistic		,134	,146	,087
Asymp. Sig. (2-tailed)		,000[c]	,000[c]	,088[c]

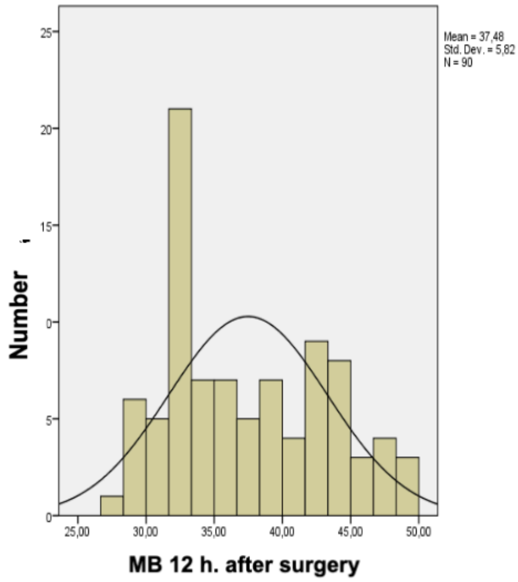
a. Test distribution is Normal.

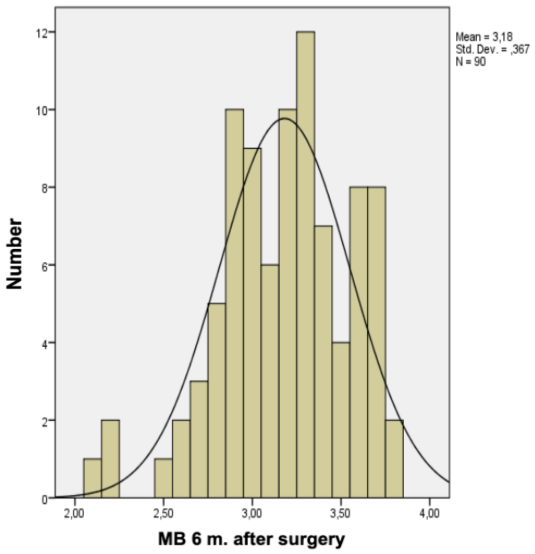
b. Calculated from data.

c. Lilliefors Significance Correction.

Diagram 6: Histograms of isoenzyme MB at different time points of the study

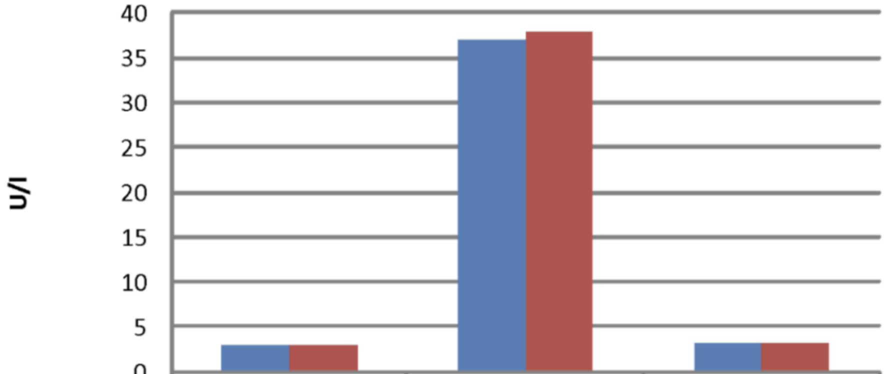






The mean values of isoenzyme MB of the two groups studied in dynamics are presented in Figure 7:

Diagram 7: Mean values of isoenzyme MB in the experimental and control groups



	12 h. before surg.	12 h. after surg.	6 m. after surg.
Control	2,91	37	3,24
TMZ	2,95	37,95	3,12

Preoperative values did not differ from normal and were the same for both groups. In both groups, isoenzyme MB values increased sharply at the 12th postoperative hour and reached values close to baseline at the 6th month, with no significant difference for control and treatment groups.

With the results thus presented, we report no effect of trimetazidine use on isoenzyme MB values at all time points in the study. We can conclude that the metabolic effects of TMZ do not influence the cellular damage expressed by the elevated isoenzyme MB levels. To be sure of the reliability of this conclusion, we used a Mann-Whitney U test and Independent samples T- test for comparison.

We compared isoenzyme MB values in the two groups using the Mann-Whitney U test. There was no statistically significant difference between the two groups at all time points (Table 6).

Table 6. Comparison of isoenzyme MB between TMZ and control group (results of Mann-Whitney U test).

	MB 12 h before surgery	MB 12 h after surgery	MB 6 months after surgery
Mann-Whitney U	917,500	916,500	879,500
Wilcoxon W	1952,500	1951,500	1914,500
Z	-,776	-,777	-1,078
Asymp. Sig. (2-tailed)	,438	,437	,281

We calculated the change in isoenzyme MB (%) from 12 hours after surgery to 6 months after surgery using the following formula:

Change in MB= ((value of MB 6 months after discharge - value of MB 12 hours after surgery)/value of MB 12 hours after surgery))*100

The distribution of this quantity was normal and therefore we applied Independent samples T- test to compare the variation in the two groups (Table 7)

Table 7. Comparison of isoenzyme MB variation in control and TMZ groups.

	Groups	N	Mean	Std. Deviation	Std. Error Mean	t	P-value
Modification of isoenzyme MB	Control	45	-91,0074	1,80266	0,26	1,727	0,08
	TMZ	45	-91,6183	1,54264	0,22		
Average difference	0,61				95% confidence interval [-0.091; 1.31]		

The variation of isoenzyme MB values in both groups was the same for all time points of the study

3.2. Troponin T (hs-TrT)

Troponin is part of the thin filament in the sarcomere and is involved in the calcium-dependent interaction between actin and myosin. It is a protein complex composed of three subunits : troponin C(TrC), troponin I(TrI) and troponin T(TrT), a tropomyosin-binding protein that links the troponin complex to the tropomyosin chain. Troponin is found mainly in striated muscle tissue, with the TrI and TrT subunits being cardiac-specific.

The release of troponin from the myocardium may result from the normal cell cycle such as apoptosis, release from the cells of troponin-degradation products, reversible increase in cell membrane permeability, cardiomyocyte edema leading to the formation and release of membrane vacuoles, and myocardial necrosis. These normal baseline levels increase rapidly and can be reported in the laboratory in myocardial injury after cardiac surgery[124] (The Evolution and Future Direction of The Cardiac Biomarker).

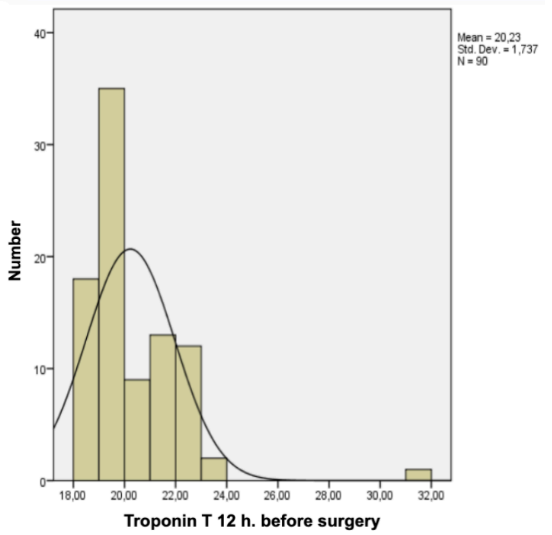
Using Kolmogorov-Smirnov test, we analyzed the distribution of **troponin T (ng/l)** 12 hours before surgery; 12 hours after surgery; 6th month after surgery (Table 8) . We found different than normal distribution for research troponin T 12 hours before surgery and 6 months after surgery :

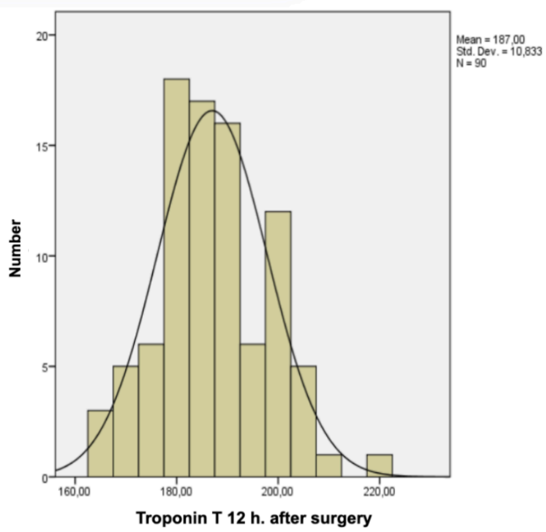
Table 8. Study of **troponin T** distribution at different time points studied (results of Kolmogorov-Smirnov test)

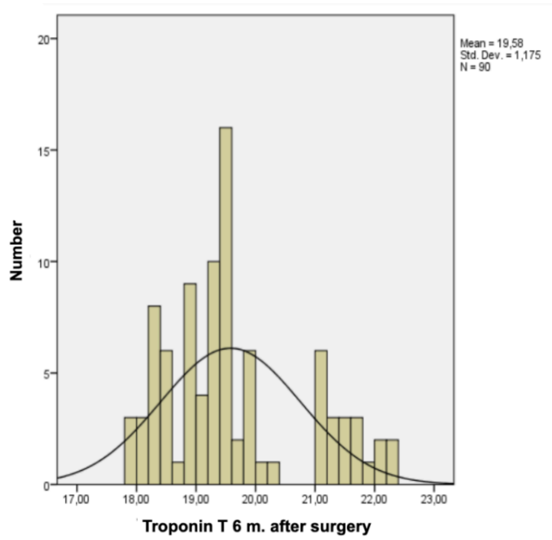
One-Sample Kolmogorov-Smirnov Test				
		hs-TrT 12hrs preOP	hs-TrT 12hrs postOP	hs-TrT 6 months postOP
N		90	90	90
Normal Parameters[a,b]	Mean	20,2278	187,0000	19,5789
	Std. Deviation	1,73659	10,83274	1,17478
Most Extreme Differences	Absolute	,164	,080	,193
	Positive	,164	,080	,193
	Negative	-,133	-,056	-,109
Test Statistic		,164	,080	,193
Asymp. Sig. (2-tailed)		,000[c]	,200[c,d]	,000[c]

a. Test distribution is Normal.
b. Calculated from data.
c. Lilliefors Significance Correction.
d. This is a lower bound of the true significance.

Diagram 8: Histograms of troponin T at different time points of the study



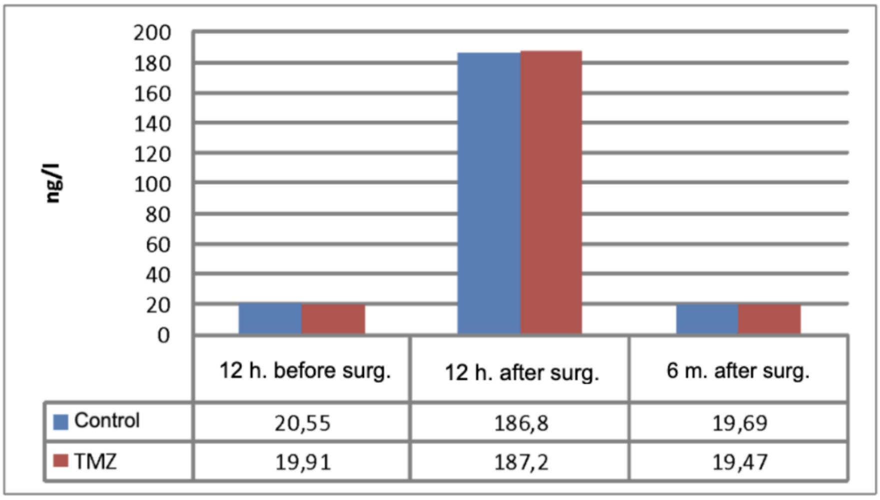




We compared the mean values at each time point in the study - at the 12th hour preoperatively, the 12th hour postoperatively, and at the 6th month postoperatively in both control and trimetazidine-treated groups.

The mean values of troponin T in the two groups studied in dynamics are presented in Figure 9.

Diagram 9: Mean values of troponin T in dynamics in experimental and control group.



The dynamics as well as the mean troponin T values in the control and treatment groups showed no significant differences. The reliability of these results was confirmed by the Mann-Whitney U test for variables with a distribution different from normal, as well as by the comparison using the Independent samples T - test.

We compared troponin T values in the two groups using the Mann-Whitney U test for variables with a distribution different from normal, measurements 12 h before surgery and 6 months after surgery (Table 9).

Table 9. Comparison of troponin T between TMZ and control group (Mann-Whitney U test results).

	Troponin T 12 hrs before op.	Troponin t 6 m. after op.
Mann-Whitney U	797,00	892,50
Wilcoxon W	1832,00	1927,50
Z	-1,74	-0,97
Asymp. Sig. (2-tailed)	0,082	0,332

The values measured 12 hours after surgery were compared with independent samples T-test (Table 10). There was no statistically significant difference between the two groups at all time points.

Table 10. Comparison of troponin T between TMZ and control group (Independent samples T - test).

	Group	N	Mean	Std. Deviation	Std. Error Mean	t	P-value
Troponin T 12 h after surgery	Control	45	186,80	12,52	1,86	0,174	0,862
	TMZ	45	187,20	8,97	1,33		

We calculated the change in troponin T (in %) for the period 12 h after surgery to 6 months after surgery using the following formula: change troponin T = ((troponin T value 6 mo after discharge - troponin T value 12 h after surgery)/ troponin T value 12 h after surgery))*100

A comparison of the change in the two groups is presented in Table 11.

Table 11. Comparison of troponin T change in control and TMZ groups.

	Groups	N	Mean	Std. Deviation	Std. Error Mean	t	P-value
Troponin T change	Control	45	-89,41	1,04	0,15	0,84	0,403
	TMZ	45	-89,57	0,82	0,12		
Average difference	0,17				95% confidence interval [-0.23; 0.56]		

Thus, the results showed normal serum troponin T dynamics for cardiac surgery patients with ECC and aortic valve replacement. With basal levels close to normal, a sharp rise immediately after surgery followed and again a fall to basal levels at the 6th month in both groups. Trimetazidine administration did not affect troponin T levels in the treatment group: mean values at all time points did not differ from the control group. We can conclude that the metabolic effects of TMZ did not influence the cellular damage expressed by the elevated troponin T levels.

3.3 Malondialdehyde (MDA) (nmol/ml)

MDA is a product of the peroxidation of polyunsaturated fatty acids with two or more methylene bonds. Its amount in serum correlates with a rise in oxidative stress. Measurement of its concentration in the blood gives a quantitative estimate of oxidative stress per se.

Using the Kolmogorov-Smirnov test, we analyzed the distribution of MDA 12 hours before surgery; 12 hours after surgery; 1 month after surgery; 6 months after surgery (Table 3.3.1) . We found a different than normal distribution for isoenzyme MB at all time points of study (Table 12):

Table 12. Analysis of the distribution of MDA at different points in the study (results of Kolmogorov-Smirnov test)

One-Sample Kolmogorov-Smirnov Test

		MDA 12 hrs before op.	MDA 12 p.m. op.	MDA 1 month after op.	MDA 6 months after op.
N		90	90	90	90
Normal Parameters[a,b]	Mean	234,3111	285,4889	248,4778	220,9889
	Std. Deviation	13,26959	9,67360	22,42853	10,83429
Most Extreme Differences	Absolute	,145	,077	,255	,188
	Positive	,145	,077	,255	,188
	Negative	-,124	-,070	-,196	-,119
Test Statistic		,145	,077	,255	,188
Asymp. Sig. (2-tailed)		,000[c]	,200[c,d]	,000[c]	,000[c]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

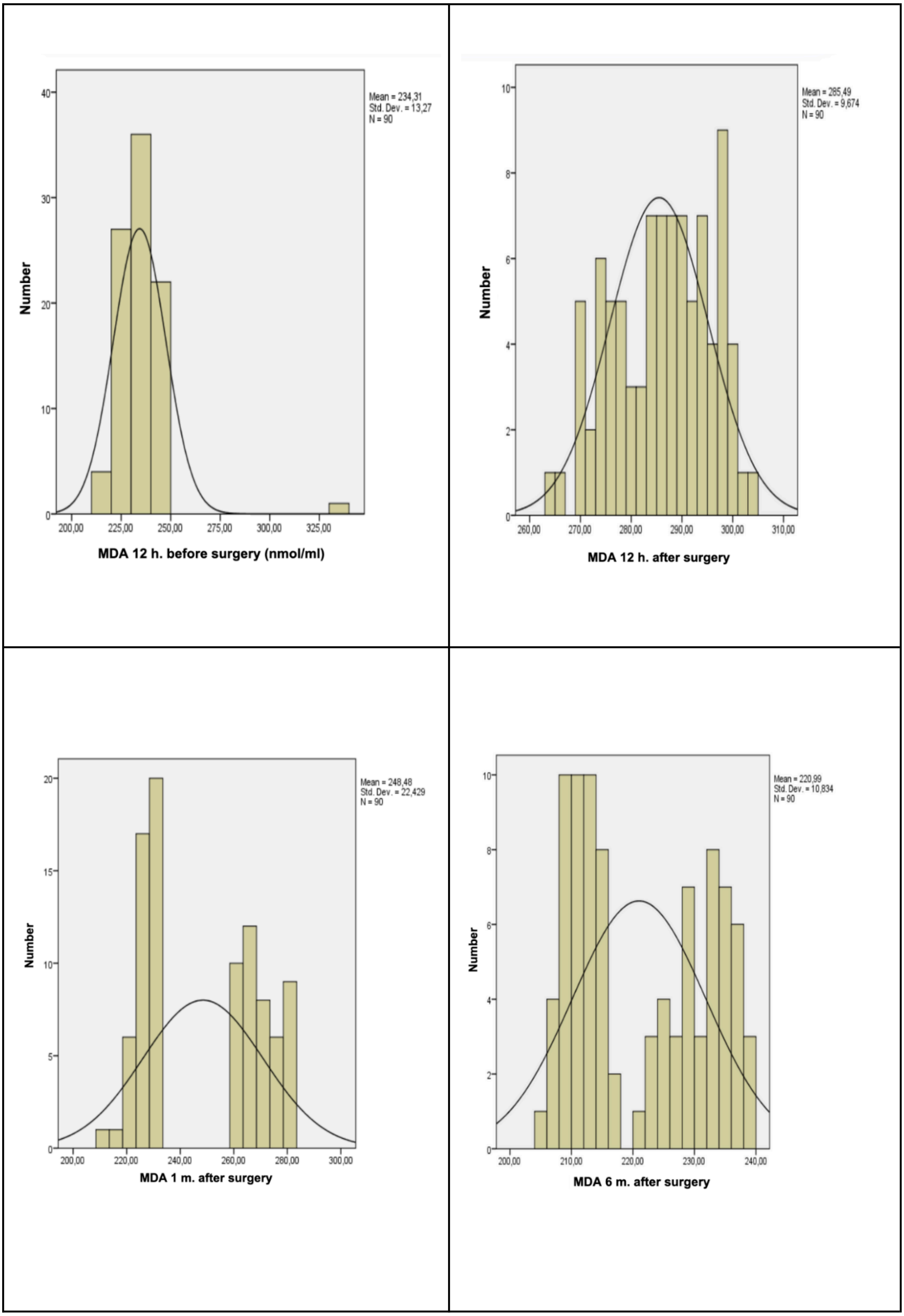
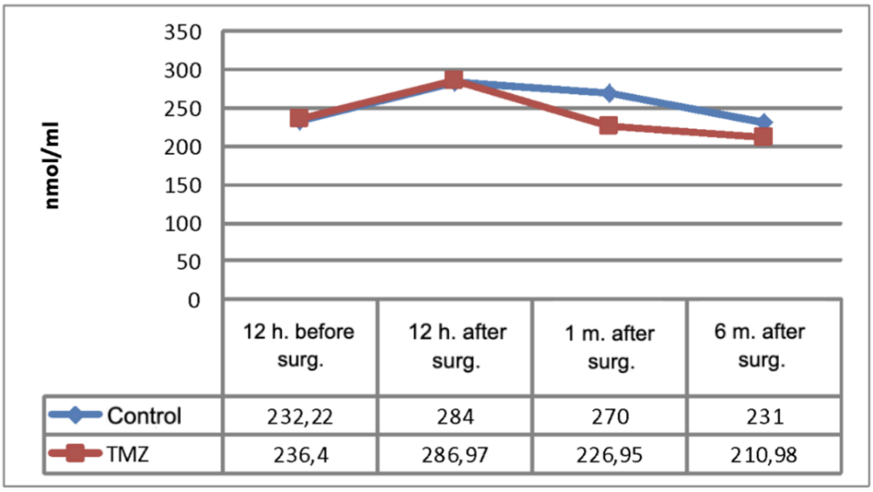


Chart 10: Histograms of MDA at different points in the study

The mean MDA values of the two groups studied in dynamics are presented in Figure 11.

Diagram 11: Mean values of MDA in dynamics in experimental and control group



	12 h. before surg.	12 h. after surg.	1 m. after surg.	6 m. after surg.
Control	232,22	284	270	231
TMZ	236,4	286,97	226,95	210,98

We compared the MDA values in the two groups using the Mann-Whitney U test. We found a statistically significant difference between the two groups at all time points except 12 h after surgery (Table 13).

Although small, the group of patients treated with trimetazidine started preoperatively with higher mean MDA values, and the difference was statistically significant. At the 12th post-operative hour and around the 7th after the first administration of trimetazidine, serum MDA levels increased the most, equalizing in the two groups, with no statistically significant difference in the mean value. At the 1st month, a decrease in its serum concentration follows, for the treatment group it is significantly more and and values below preoperative are reached, For the control group this decrease is much less significant. At the 6th postoperative month, the trend of decreasing MDA levels remained in both groups, with the control group reaching preoperative serum concentrations and the treatment group reaching mean values well below preoperative.

The reliability of these results was also confirmed by Mann-Whitney U test for variables with a distribution different from normal, as well as by comparison using Independent samples T - test.

Table 13. Comparison of MDA between TMZ and control group (Mann-Whitney U test results).

	MDA 12 hrs before op.	MDA 12 p.m. op.	MDA1 m. after op.	MDA 6 m. post op.
Mann-Whitney U	574,500	841,500	,000	,000
Wilcoxon W	1609,500	1876,500	1035,000	1035,000
Z	-3,537	-1,381	-8,176	-8,179
Asymp. Sig. (2-tailed)	,000	,167	,000	,000

We calculated the change in MDA (%) between 12 hours after surgery and 6 months after surgery using the following formula:

Change in MDA= ((MDA value 6 months post discharge - MDA value 12 hours post surgery)/ MDA value 12 hours post surgery))*100

The distribution of this quantity was normal and therefore we applied Independent samples T- test to compare the variation in the two groups (Table 14).

Table 14. Comparison of MDA change in control and TMZ groups.

	Groups	N	Mean	Std. Deviation	Std. Error Mean	t	P-value
Amendment of the MDA	Control	45	-22,9607	3,99593	,59568	13.76	P<0.0001
	TMZ	45	-36,0545	4,97370	,74144		
Average difference	13.09				95% confidence interval [11,20; 14,98]		

Comparison of MDA values by sex.

We examined malondialdehyde values by sex. The comparison is presented in Table 15.

Table 15. Comparison of mean MDA values by sex.

	Gender	N	Mean	SEM	t	P
MDA 12 hrs before op.	men	67	233,23	,97	0.87	0.39
	women	23	237,43	4,69		
MDA 12 p.m. op.	men	67	285,17	1,19		
	women	23	286,39	1,97	0.51	0.61
MDA1m. after op.	men	67	247,91	2,79		
	women	23	250,13	4,49	0.41	0.68
MDA 6 m. post op.	men	67	221,04	1,34	0.08	0.93
	women	23	220,82	2,17		

There was no statistically significant gender difference in mean MDA values for the control and treatment groups.

We stratified the study patients by sex and analyzed the difference in mean MDA levels separately in men and women. The results are presented in Table 16 and Table 17, respectively.

Table 16. Comparison of mean MDA values in men between control and treatment groups.

	Groups	N	Mean	SEM	t	P
MDA 12 hrs before op.	Control	33	229,03	1,31	4.95	<0.0001
	TMZ gr.	34	237,32	1,04		
MDA 12 p.m. op.	Control	33	284,06	1,78	0.92	0.391
	TMZ gr.	34	286,26	1,60		
MDA1 m. after op.	Control	33	270,15	1,19	30.23	<0.0001
	TMZ gr.	34	226,32	0,82		
MDA 6 m. post op.	Control	33	231,42	0,86	20.87	<0.0001
	TMZ gr.	34	210,97	0,48		

Table 17. Comparison of mean MDA values in women between control and treatment group.

	Groups	N	Mean	SEM	t	P
MDA 12 hrs before op.	Control	12	241,00	8,85	0.78	0.44
	TMZ gr.	11	233,54	2,14		
MDA 12 p.m. op.	Control	12	283,83	2,78	1.37	0.18
	TMZ gr.	11	289,18	2,68		
MDA1 m. after op.	Control	12	269,58	2,18	16.46	<0.0001
	TMZ gr.	11	228,90	0,96		
MDA 6 m. post op.	Control	12	229,83	1,41	11.12	<0.0001
	TMZ gr.	11	211,00	0,93		

There was a significant significant difference in MDA levels between the TMZ group and the control group in both men and women 1 month and 6 months after surgery. There was also a difference in men 12 hours before surgery, and MDA levels were higher in the treatment group at this measurement.

Influence of age.

We analyzed the effect of age on malondialdehyde values. For this purpose, we grouped the studied patients into two groups, those aged 68 years and older. We chose the age limit of 68 years according to definition adopted by WHO in 2012 from an article published in the International Journal of Epidemiology , where people over the age of 68 years are considered as adults [116] We compared the MDA values in the two age groups (Table 18).

Table 18. Comparison of mean MDA values by age groups.

	Age gr.	N	Mean	SEM	t	P
MDA 12 hrs before op.	<=68 г.	64	235,10	1,84	0,89	0,37
	> 68 г.	26	232,34	1,67		
MDA 12 p.m. op.	<=68 г.	64	285,26	1,21	0,32	0,733
	> 68 г.	26	286,03	1,89		
MDA1 m. after op.	<=68 г.	64	248,35	2,83	0,078	0,938
	> 68 г.	26	248,76	4,35		
MDA 6 m. post op.	<=68 г.	64	221,28	1,32	0,400	0,690
	> 68 г.	26	220,26	2,25		

There was no statistically significant difference between the two age groups.

We obtained similar results for the treatment group only (Table 19).

Table 19. Comparison of mean MDA values by age groups in TMZ patients.

	Art.	N	Mean	SEM	t	P
MDA 12 hrs before op.	<=68 г.	32	236,15	1,11	0,391	0,698
	> 68 г.	13	237,00	1,98		
MDA 12 p.m. op.	<=68 г.	32	286,62	1,63	0,398	0,693
	> 68 г.	13	287,84	2,63		
MDA1 m. after op.	<=68 г.	32	226,71	0,86	0,538	0,594
	> 68 г.	13	227,53	1,10		
MDA 6 m. post op.	<=68 г.	32	211,43	0,45	1,732	0,090
	> 68 г.	13	209,84	0,89		

3.4. Indicators related to hospital stay

We examined the following indicators related to patients' hospital stay:

- stay in intensive care (in hours);
- total hospital stay (in days)

There was no statistically significant difference between the control and TMZ groups for the ICU stay. Regarding the total hospital stay, there was a trend (with marginal statistical significance) for less hospital stay in the TMZ group (Table 20).

Table 20. Comparison of ICU stay and total hospital stay between control group and TMZ group.

	Groups	N	Mean	Std. Deviation	Std. Error Mean	t	P
Resuscitation stay (h)	Control	45	23,31	2,66	0,39	0.264	0.792
	TMZ	45	23,46	2,91	0,43		
Hospital stay (days)	Control	45	7,17	0,71	0,10	1.85	0.067
	TMZ	45	6,86	0,86	0,12		

The mean length of stay in intensive care was almost the same for both groups - 23.31 hours for the control group and 23.46 hours for the treatment group - the difference was nonsignificant.

The administration of trimetazidine had an impact on hospital stay, not affecting the recovery time in the intensive care unit, but shortening the total hospital stay (7.17 days for the control versus 6.86 days for the treatment group at p 0.067).

3.6. Correlation analysis

We analyzed the correlation between MDA at all study time points and the parameters ICU stay, total hospital stay, clamp time, ECC duration, and total blood loss in the control and TMZ groups. The calculated Spearman correlation coefficients for the control and TMZ groups are presented in Table 21 and Table 22, respectively.

Table 21. Correlations in the control group (Spearman correlation coefficient)

			Stay in intensive care	Hospital stay	Clamping time	Duration of the ECC	Total blood loss
Spearman's rho	MDA 12 hrs before op.	Correlation coefficient	0,150	-0,067	-0,030	-0,082	0,159
		Sig. (2-tailed)	0,326	0,663	0,846	0,594	0,297
		N	45	45	45	45	45
	MDA 12 p.m. op.	Correlation coefficient	-0,007	0,055	0,580**	0,581**	0,079
		Sig. (2-tailed)	0,963	0,717	0,0001	0,0001	0,606
		N	45	45	45	45	45
	MDA1 month after op.	Correlation coefficient	0,104	-0,164	0,839**	0,174	-0,007
		Sig. (2-tailed)	0,495	0,282	0,0001	0,253	0,965
		N	45	45	45	45	45
	MDA 6 months after op.	Correlation coefficient	0,062	-0,031	0,753**	0,256	0,039
		Sig. (2-tailed)	0,683	0,840	0,0001	0,090	0,798
		N	45	45	45	45	45

We found the following statistically significant straight correlations in the control group: MDA 12 h after surgery (r=0.58, P=0.0001); MDA 1 month after surgery (r=0.839, P=0.0001); MDA 6 months (r=0.753, P=0.0001) after surgery with claudication time, and MDA 12 h after surgery and ECC duration (r=0.581, P=0.0001).

These correlations show a significant increase in oxidative stress, by quantitative increase in MDA, with increasing aortic valve duration, at all time points examined. Such a significant increase was also observed with respect to ECC duration, but only in the measurement at the 12th hour after surgery. Thus interpreted, the data confirm the relationship between valve duration and ECC and the magnitude of oxidative stress noted in previous studies [117][118][119][120][121][122][123].

Table 22. Correlations in TMZ group (Spearman correlation coefficient)

			Stay in intensive care	Hospital stay	Clamping time	Duration of the ECC	Total blood loss
Spearman's rho	MDA 12 hrs before op.	Correlation coefficient	-0,285	0,154	0,215	0,422**	-0,120
		Sig. (2-tailed)	0,058	0,313	0,156	0,004	0,434
		N	45	45	45	45	45
	MDA 12 p.m. op.	Correlation coefficient	-0,087	-0,048	0,776**	0,071	0,143
		Sig. (2-tailed)	0,570	0,752	0,0001	0,644	0,350
		N	45	45	45	45	45
	MDA1 month after op.	Correlation coefficient	-0,113	-0,379*	-0,278	0,035	-0,208
		Sig. (2-tailed)	0,461	0,010	0,065	0,820	0,169
		N	45	45	45	45	45
	MDA 6 months after op.	Correlation coefficient	-0,162	0,120	-0,159	0,228	0,291
		Sig. (2-tailed)	0,286	0,432	0,296	0,132	0,053
		N	45	45	45	45	45

In the TMZ group, we found a statistically significant moderate direct correlation between MDA 12 h preoperatively and ECC duration (r=0.422, P=0.004) - here we can conclude that higher levels of oxidative stress preoperatively reflect on the reperfusion time required for the myocardium to recover after valve replacement. The statistically significant longer ECC times, with identical valve times and standard operative technique, can only be attributed to the longer reperfusion time, although this parameter was not sought and noted in our study. No such correlation was observed in the control group, but there the mean level of MDA preoperatively was lower, preventing such a correlation from manifesting itself.

There was no correlation between ECC time in the treatment group and MDA levels at the 12th postoperative hour, as was present in the control group. Already here we can assume an effect of TMZ neutralizing the peak levels of oxidative stress.

There was a statistically significant strong direct correlation between MDA 12 h after surgery and claudication time (r=0.776, P=0.0001). This result correlated with the results in the control group. There was no correlation between clamp time and MDA levels at 1st and 6th month after surgery, unlike the control group. Here, we can conclude that the administration of trimetazidine affects more the high levels of oxidative stress and this leads to a "smoothing" of MDA values.

We observed a statistically significant moderate inverse correlation between MDA 1 month after surgery and hospital stay (r=-0.379, P=0.01). No such relationship was observed in the control group, where different levels of oxidative stress did not result in differences in hospital length of stay.

There was no correlation between malondialdehyde levels and resuscitation stay, and malondialdehyde levels and total blood loss for both groups.

In contrast to the control group, in the trimetazidine-treated group increased aortic valve-clamping time correlated with an increase in oxidative stress only in the study period of the 12th hour after surgery. The lack of correlations in the other time periods compared with the control group indicates the presence of a TMZ effect of significant magnitude. We can note the same with respect to the duration of ECC. We can conclude that the application of

TMZ „attenuates" the negative effects of increased aortic valve duration and ECC. The reduction of oxidative stress in the treatment group resulted in a reduction of total hospital stay, but did not affect the length of stay in intensive care.

At the 12th postoperative hour, the increase in MDA levels in both groups correlated with aortic valve duration, and this correlation was slightly more pronounced for the treatment group.

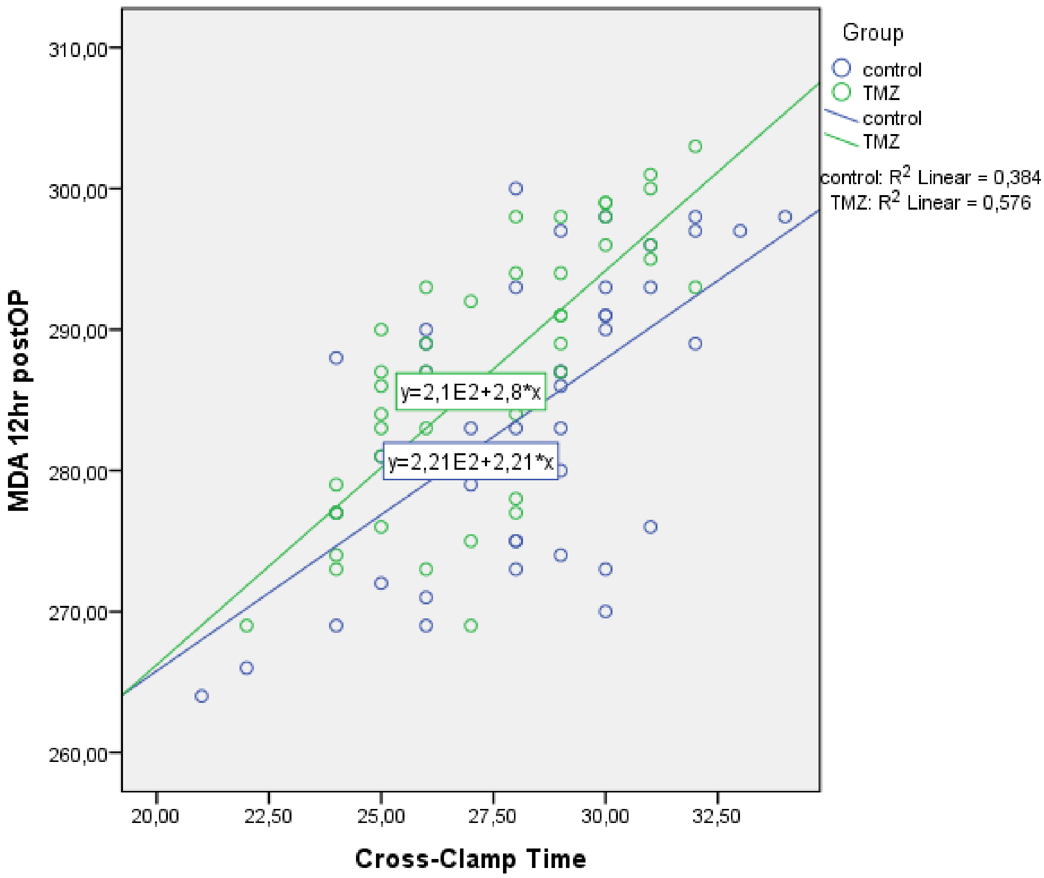


Diagram 12: Correlation between claudication time and MDA 12 h postoperatively in the control and TMZ groups.

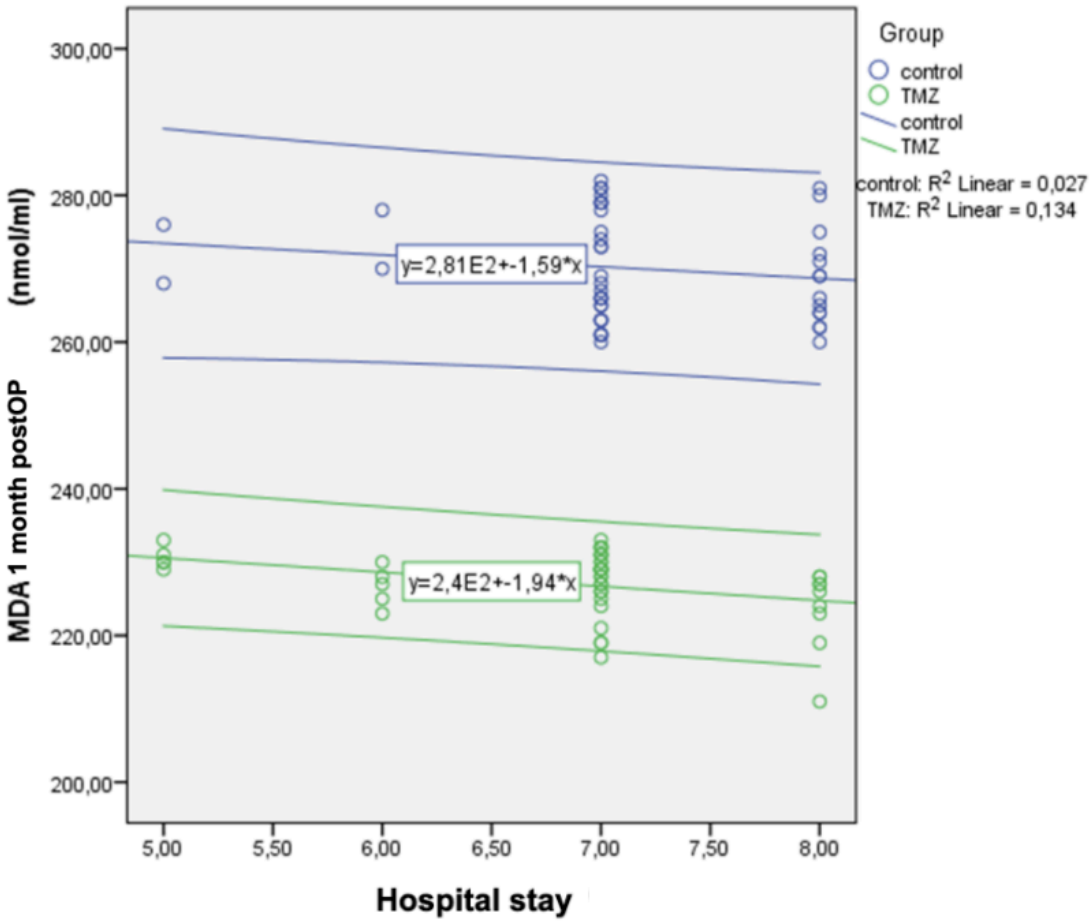


Figure 13: Correlation between hospital stay and MDA 1 month after surgery in the control and TMZ groups.

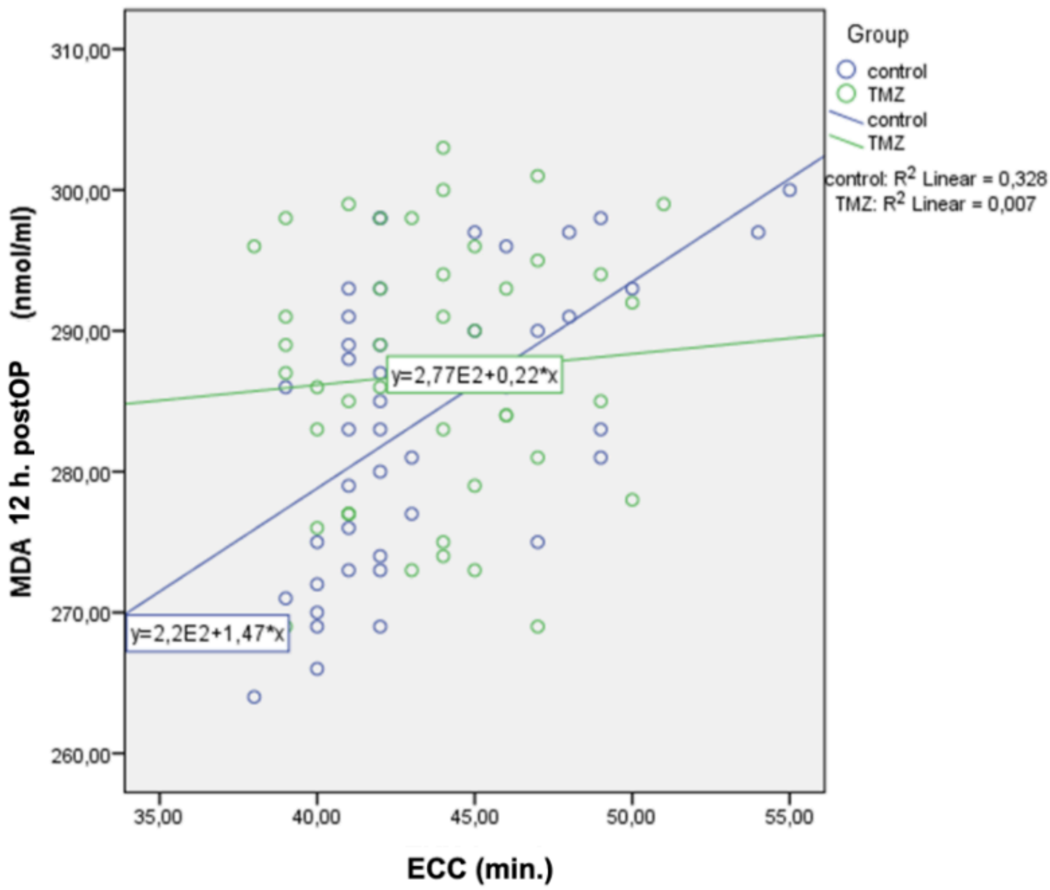


Diagram 14: Correlation between extracorporeal circulation and MDA 12 h after surgery in the control and TMZ groups.

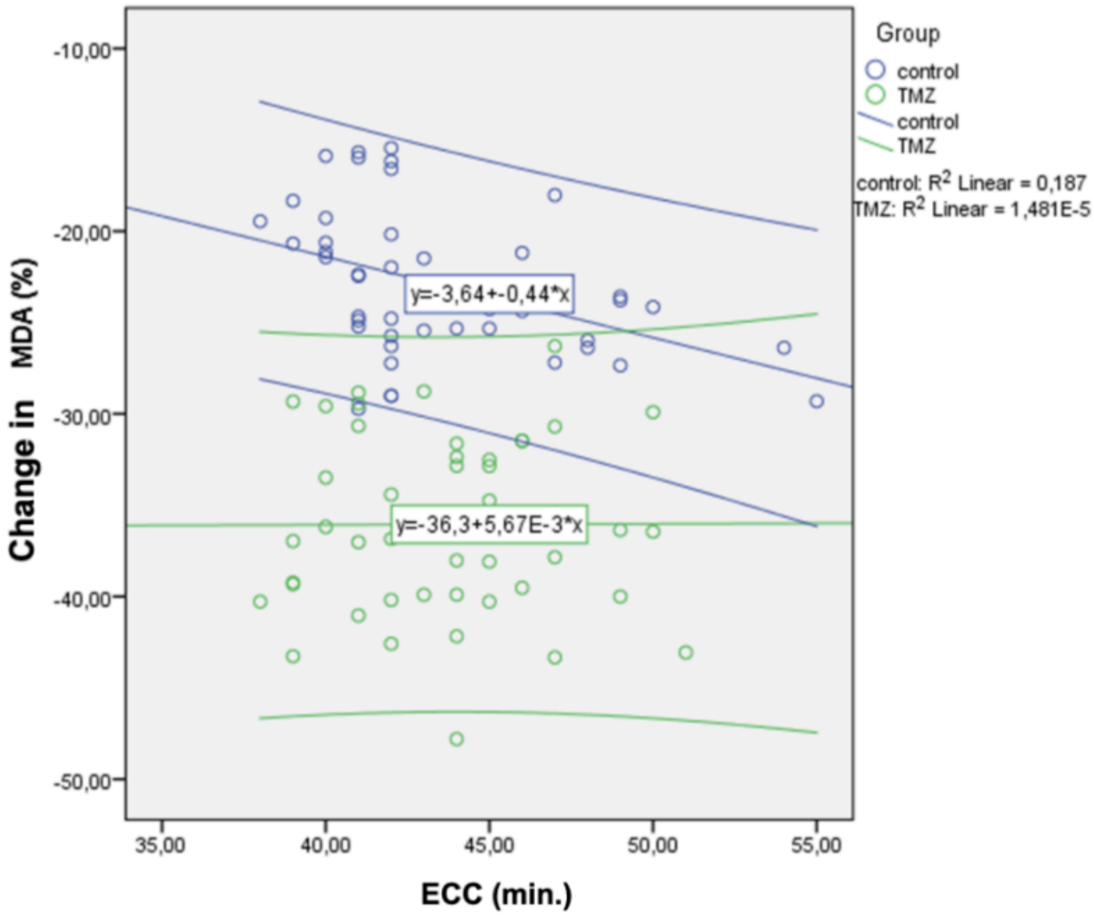


Diagram 15: Correlation between extracorporeal circulation and change in MDA (6 months postoperative versus 12 h postoperative) in the control and TMZ groups.

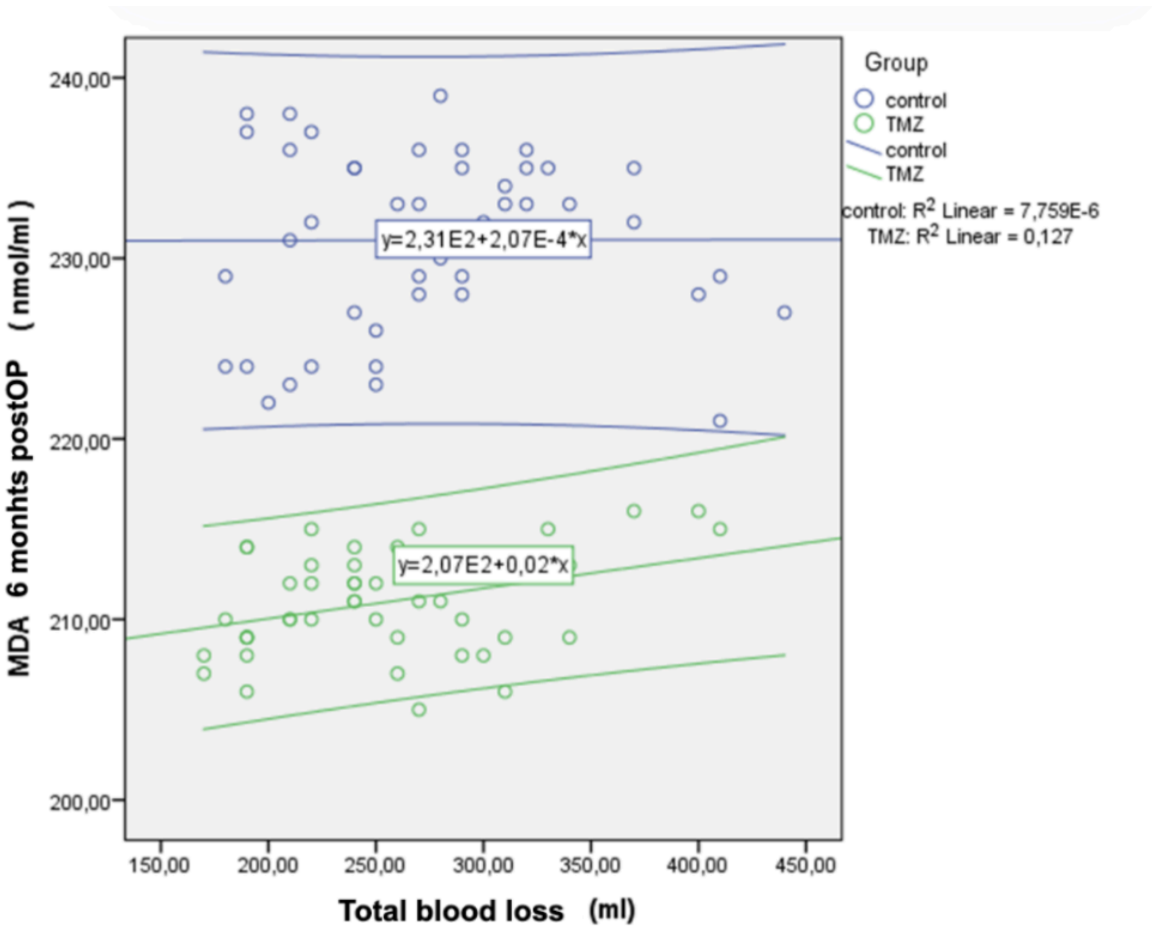


Figure 16: Correlation between total blood loss and MDA 6m postoperatively in the control and TMZ groups.

2. Analysis of sonographic parameters

As a criterion for objectification of left ventricular function, we adopted the ejection fraction (EF) measured sonographically by Simpson's method.

Using the Kolmogorov-Smirnov test, we analyzed the distribution of the studied echographic parameters (Table 23). We found a different from normal distribution for the ejection fraction - FI (measured in %) at all time points of the study:

- echocardiography FI 12 hours before surgery (P<0.0001)
- echocardiography FI 1 hour after surgery (P=0.005)
- echocardiography FI at discharge (P<0.0001)
- echocardiography FI at 6th month after surgery (P=0.04)

Table 23. Investigation of the distribution of FI at different moments of the investigated (results of Kolmogorov-Smirnov test)

One-Sample Kolmogorov-Smirnov Test

		Echo EF 12hrs preOP	Echo EF 1hr postOP	Echo EF on discharge	Echo EF 6 months postOP
N		90	90	90	90
Normal Parameters[a,b]	Mean	44,6889	44,2667	47,7222	54,1111
	Std. Deviation	2,68430	2,86317	2,86799	3,12455
Most Extreme Differences	Absolute	,146	,115	,133	,096
	Positive	,146	,115	,133	,096
	Negative	-,085	-,085	-,083	-,089
Test Statistic		,146	,115	,133	,096
Asymp. Sig. (2-tailed)		,000[c]	,005[c]	,000[c]	,040[c]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

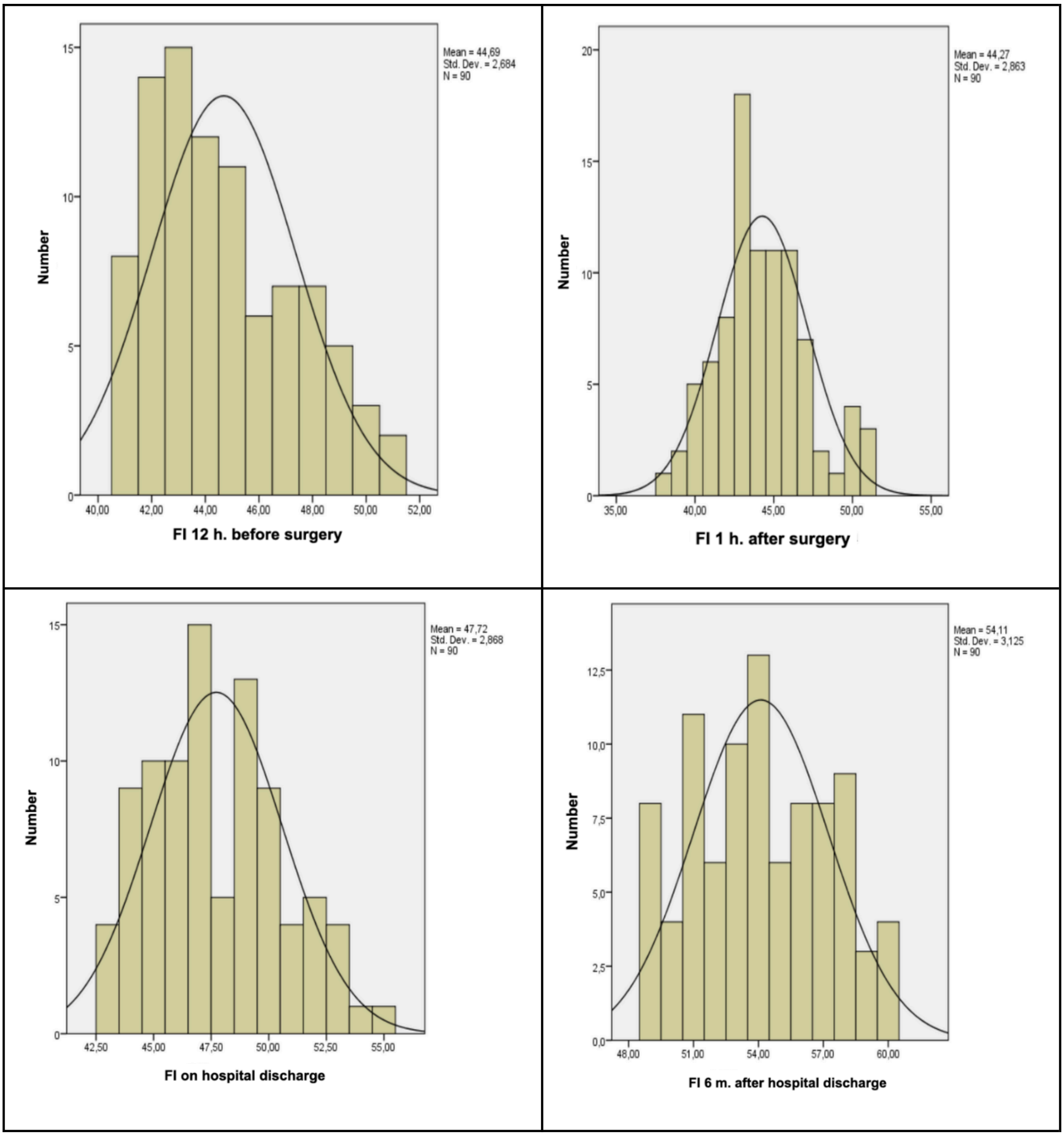


Chart 17: Histograms of FI at different points in the study

The mean FI values of the two groups studied in the dynamics are presented in Figure 18.

In both groups, there was a slight decrease in FI at the first hour after surgery, followed by an increase at discharge and at the 6th month, with the same dynamics for both groups.

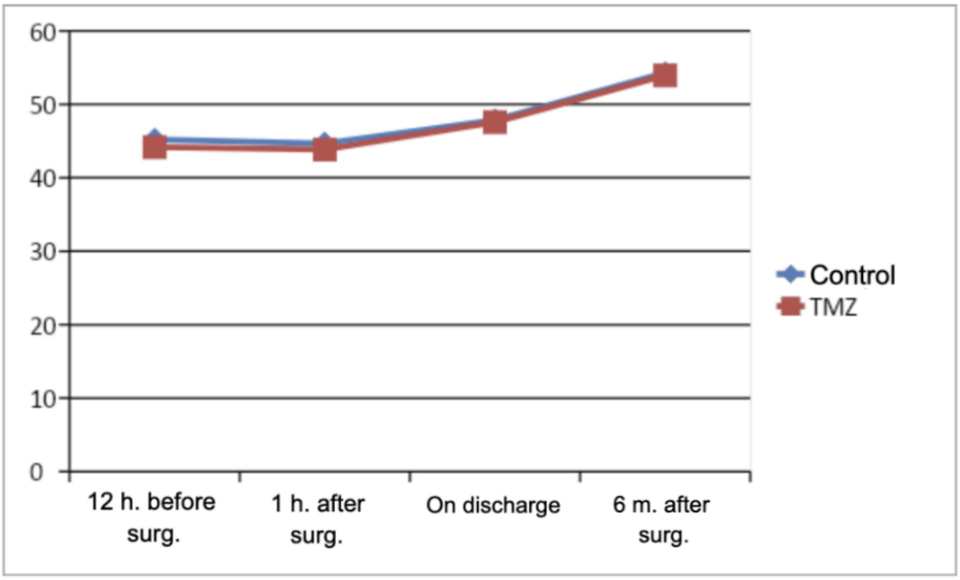


Chart 18: Mean values of FI in dynamics in experimental and control group

The reliability of these results was also confirmed by the Mann-Whitney U test for variables with a distribution other than normal, as well as by the Independent samples T-test.

We compared the FI values in the two groups using the Mann-Whitney U test. There was no statistically significant difference between the two groups at all time points (Table 24).

Table 24. Comparison of FI between TMZ and control group (Mann-Whitney U test results).

	FI 12 hrs. before op.	FI 1 hrs. after op.	PHI at discharge	FI 6 m. after op.
Mann-Whitney U	783,500	911,000	960,500	963,000
Wilcoxon W	1818,500	1946,000	1995,500	1998,000
Z	-1,862	-,825	-,422	-,401
Asymp. Sig. (2-tailed)	,063	,409	,673	,688

We calculated the change in FI (%) 1 hour after surgery until the time of patient discharge using the following formula:

Change in PHI= ((PHI value at discharge - PHI value 1 h after surgery)/PHI value 1 h after surgery))*100

The distribution of this quantity was normal and therefore we applied Independent samples T- test to compare the variation in the two groups (Table 25).

Table 25. Comparison of the change in FI in the control and TMZ groups.

	Group	N	Mean	Std. Deviation	Std. Error Mean	t	P-value
Change in FI	control	45	7,2392	3,14566	,46893	1,58	0,117
	TMZ	45	8,5862	4,76110	,70974		
Average difference	-1,35				95% confidence interval [-3.037; 0.343]		

We found a statistically significant inverse correlation between ejection fraction 12 h before surgery and MDA 12 h after surgery in the TMZ group (r=-0.304; P=0.042).

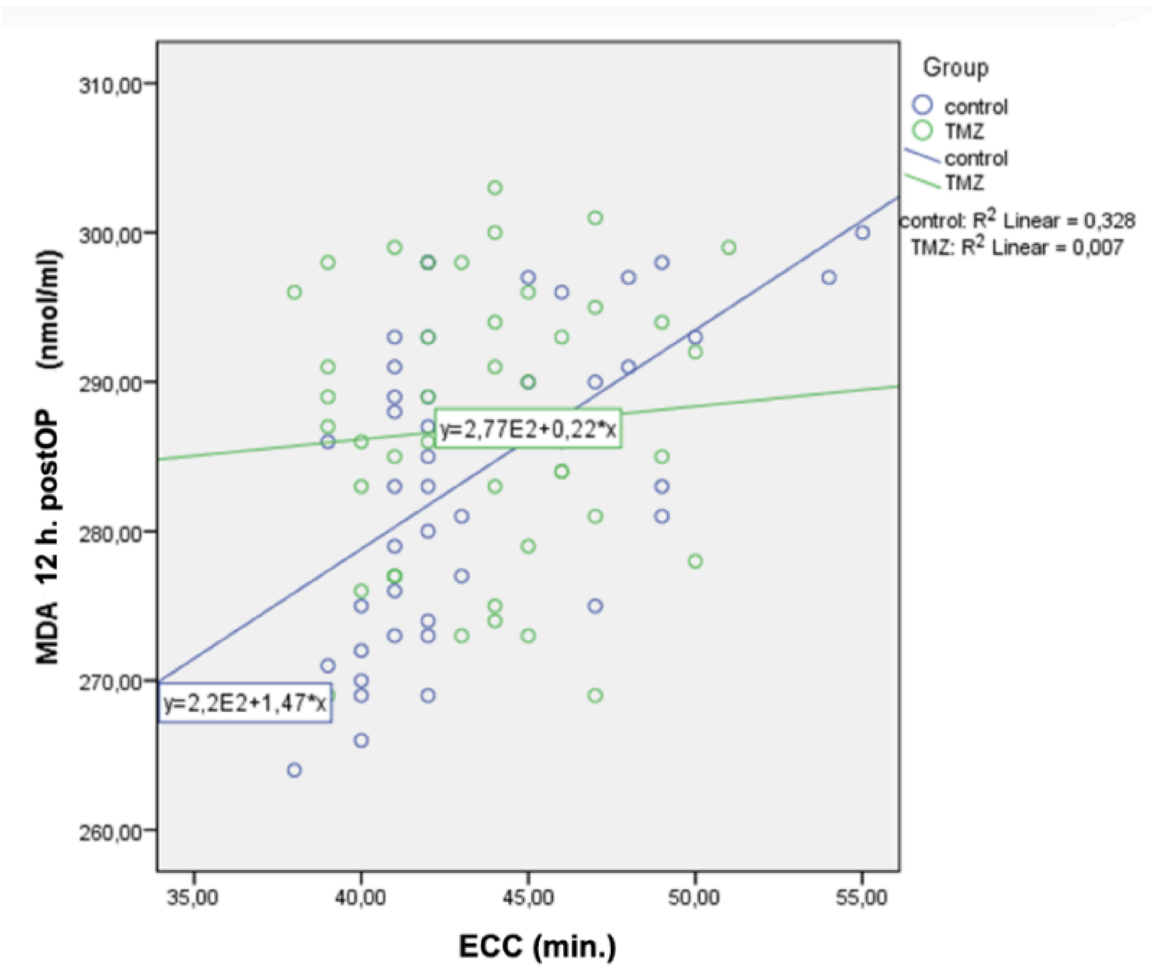


Diagram 19: Correlation between echocardiographic ejection fraction 12 h before surgery MDA 12 h after surgery in the TMZ group.

V. Discussion

Ischaemic heart disease (IHD) is a socially significant, chronic progressive disease resulting from obstructive or non-obstructive atherosclerotic involvement of epicardial vessels. It is one of the leading causes of impaired quality of life and cardiovascular mortality.

The term ischaemic heart disease describes a group of clinical syndromes characterised by myocardial ischaemia and an imbalance between the blood supply to the myocardium and its instantaneous demand. Because the fundamental pathophysiological defect in the ischemic myocardium is inadequate perfusion, ischemia is associated not only with insufficient oxygen supply, but also with reduced nutrient availability and inadequate removal of metabolic end products. In myocardial ischemia, the consequences of oxygen deprivation cannot be separated from the effects of impaired metabolite washout.

The clinical syndromes of CHD cause more deaths, morbidity and financial burden in Western societies than any other group of diseases.

Despite progressive reductions in CHD mortality due to improved treatment strategies and preventive measures, 1 in 6 deaths in the U.S. is attributable to coronary disease. In most patients with CHD, the cause of myocardial ischemia is a reduction in coronary blood flow due to atherosclerotic coronary artery disease.

The manifestations of CHD depend on the duration, severity and acuteness of ischemic episodes. Sudden critical reduction in coronary blood flow is the primary mechanism in acute coronary syndromes (ACS), a spectrum of clinical conditions that includes unstable angina, non-ST-elevation myocardial infarction and ST-elevation myocardial infarction.

In contrast, in the chronic form of CHD, the presence of blood flow-limiting coronary lesions limits the heart's ability to increase its blood supply in response to the myocardium's increased need for oxygen, leading to the development of stable angina pectoris.

The heart is an aerobic organ that derives most of its energy from mitochondrial respiration and oxidative phosphorylation. Myocardial ischemia occurs when the energy requirements of the myocardium exceed the energy-generating capacity of the mitochondria because of a deficient oxygen supply and the cardiomyocytes switch to anaerobic glycolysis as a means of generating energy. Anaerobic glycolysis is an inefficient source of energy and the capacity of glycolytic enzymes is limited; therefore, energy production from anaerobic glycolysis cannot compensate for the loss of oxidative phosphorylation and results in a negative myocardial energy balance with progressive loss of high-energy phosphate reserves. Furthermore, hydrolysis of adenine nucleotides and generation of lactic acid leads to a progressive increase in the production of reactive oxygen species and acidic products in the ischemic myocardium. It is these that cause the expansion of the zone of injury.

The treatment of CHD is medical, interventional and surgical. In the majority of cases, the surgical treatment is classical bypass surgery using extracorporeal circulation (ECC) and aortic valve replacement.

Cardiac surgery using ECC is associated with systemic inflammation, ischemia-reperfusion (IR) injury, and surgical trauma, which are strong stimulants of RNOS generation leading to increased oxidative stress during and after surgery. Increased RNOS production is observed in the reperfused ischemic heart after cardioplegic myocardial arrest during surgery, leading to deterioration of atrial and ventricular function, expressed by postoperative ventricular fibrillations (POAF) and ejection fraction reduction.

As a quantitative marker of oxidative stress levels, we use the serum concentration of malondialdehyde (MDA).The main source of MDA in biological samples is the peroxidation of polyunsaturated fatty acids with two or more methylene double bonds. Lipid peroxidation is an important component of oxidative stress. Lipid peroxidation alters membrane fluidity and permeability, sometimes irreversibly. Lipids in cell membranes, especially polyunsaturated fatty acids (PUFA), are susceptible to attack by various RNOS, such as hydroxyl radicals and peroxyhydrates [17].Typically, damage under these conditions is a multistep sequence of events beginning with RNOS-mediated hydrogen abstraction from the lipid molecule, creating a fatty acid radical, which itself abstracts a hydrogen atom from the adjacent lipid molecule, creating another fatty acid radical. The reaction formed is self-sustaining and once formed does not need the presence of RNOS. The amount of MDA correlates with the growth of the reaction.

As indicators of the degree of myocardial damage, we examined the levels of the CF isoenzyme MB, TrT, and the change in left ventricular ejection fraction.

The CF isoenzyme of CPK is used as a specific indicator of myocardial injury, including in the postoperative period in cardiac surgery. Its amount in serum increases with cardiomyocyte cell death and correlates with lesion extent.

Troponin T is a biomarker that is cardiospecific. Its amount in serum increases with the release of troponin from the myocardium, which may result from normal cell cycle such as apoptosis, release from cells of troponin-degradation products and myocardial necrosis. It is in myocardial necrosis that normal basal levels rise rapidly and can be read in the laboratory in the first hours of myocardial injury after cardiac surgery [124]. Troponin T may also be a marker of ischemia alone, when its levels rise without cell necrosis having occurred, due to reversible disturbances in cell metabolism, the expression of which is an increase in cell membrane permeability and cardiomyocyte edema leading to the formation and release of membrane vacuoles.

Left ventricular ejection fraction measured ultrasonographically is a reliable and easily applicable method for determining the functional status of the heart muscle. The method is routinely used in cardiac surgery to determine the preoperative status of the myocardium as well as to monitor recovery in the postoperative period.

Trimetazidine (TMZ), is a drug that exhibits its anti-ischemic effect without altering coronary blood flow and cardiac contractility. TMZ reduces the use of free fatty acids as an energy source for the myocardium, resulting in an increase in glucose oxidation that is more efficient and with less need for oxygen to conduct the reaction. As a result, TMZ reduces ischemic stress and improves the heart's performance during ischemia. TMZ is a widely used drug for the treatment of CHD, but there is not yet sufficient evidence-based data on its use as a means of reducing the oxidative stress that accompanies cardiac surgery.

The studies done so far follow different therapeutic models, are built on a relatively small number of patients, often with different preoperative and operative profiles. The results are controversial and do not provide a definitive answer about the benefit of TMZ administration.

1. Analysis of the study population

The study began in March 2018 and ended in April 2019, with 93 patients enrolled, of whom 3 were subsequently excluded. The overall baseline characteristics of the study group showed , that in this sample of CHD patients, the male to female ratio was 3 to 1. The sex ratio was maintained in both TMZ-treated and control groups. This ratio is explained by the nature of the different morbidity of the two sexes, due to the protective effect of female sex hormones in the fertile part of women's life. The mean age of the patients was 63.56 years, the mean age for the control group was 64.18 ± 6.54 years and for the treatment group was 62.97 ± 9.45 years. Most patients were in the age group 61-70 years for both sexes.

All patients studied had CHD - stable angina, triclonus coronary disease, with concomitant diseases arterial hypertension and diabetes mellitus on oral therapy. The mean preoperative ejection fraction for the entire study group was 44.69 ± 2.68. The final study group included 90 patients, preoperatively divided into 2 subgroups: 1st group: (n = 45) receiving trimetazidine 35 mg/ twice daily immediately after extubation and 2nd control group: (n = 45) receiving placebo. Remaining therapy was identical for all participants: acetylsalicylic acid (ASA) 100 mg/day, rosuvastatin 10 mg/day, metoprolol 50 mg/twice daily, perindopril 5 mg/day and gliclazide 60 mg/day.

All patients underwent elective cardiac surgery using median sternotomy as the operative approach, in the setting of ECC with aortic valve clamping and cardioplegic arrest achieved with cold blood cardioplegia.

The duration of ECC and clamping varied according to the time it took to perform optimal revascularization. The mean ECC time was 43.53 ± 3.89 min for the control group and 43.71 ± 3.37 min for the treatment group. The mean claudication time was 28.22 ± 2.78 for the control group and 27.42 ± 2.50 for the treatment group. The mean length of stay in the intensive care unit for the control group was 23.31 ± 2.66 hours and for the treatment group 23.46 ± 2.91 hours. The total hospital stay for the control group was 7.17 ± 0.71 days, while for the treatment group it was 6.86 ± 0.86 days. The mean total blood loss for both groups was 276.00 ± 66.24 milliliters for the control group and 255.77 ± 60.69 milliliters for the TMZ-treated group.

2. Comparison between the output characteristics of the two groups

The two main groups were compared according to their baseline characteristics in order to establish comparability between them. The results are presented in the Results section. They show that no statistically significant differences were found between the TMZ-treated groups and the control group, both in the rank of the quantitative and in the proportion of the qualitative characteristics. i.e. the groups can be compared.

3. Results

The comparison between the tracked indicators in the two groups showed the following trends. With respect to the laboratory indices CPK-MB and TrT, there was no significant difference between the trimetazidine-treated group and the control group at any point in the study. The values preoperatively did not differ from normal and were the same for both groups. In both groups, isoenzyme MB values of CPK and troponin T increased sharply at the 12th postoperative hour and reached values close to baseline at the 6th month, with no significant difference for control and treatment groups.

With the results thus presented, we report no effect of trimetazidine use on isoenzyme MB and troponin T values at all time points in the study. We can conclude that the metabolic effects of TMZ, applied in the manner adopted in the study, did not affect the cellular damage at the time points of biological sampling, the expression of which was the elevated levels of both biomarkers. This is not consistent with results presented in other studies. Turnerir et al. (1999) [104] observed the cardioprotective role of TMZ. The authors evaluated the potential myocardial protection of TMZ by measuring troponin T during CABG of thirty randomized patients with high-grade angina divided into two groups. The results showed that the TMZ group had significantly lower TnT levels than the placebo group. In this study, trimetazidine therapy in the treatment group was started three weeks before surgery and serum samples were taken only at intervals of the early postoperative period. No functional cardiac parameters were recorded. Banach et al. (2005) [107] evaluated the effect of TMZ on cardiac hemodynamic parameters and postoperative prognosis. One hundred and forty patients were included in the study, divided into two groups (placebo and TMZ). Trimetazidine therapy was given for at least two months before and after cardiac surgery. The authors monitored postoperative CK-MB levels, early postoperative ejection fraction (EF), occurrence of postoperative arrhythmias, mean intensive care unit (ICU) length of stay, and length of hospitalization. There were no significant differences in CK-MB values in the two groups. Preoperative ejection fraction was similar in both groups; however, in the postoperative period, a significant increase in FI was found in the TMZ group (p = 0.05). The authors conclude that the administration of TMZ before and after cardiac surgery may affect postoperative hemodynamic parameters. Martins et al. (2008) [103] evaluated the effect of TMZ on ischemia-reperfusion injury. The authors conducted a double-blind, placebo-controlled randomized trial on 60 patients undergoing CABG, divided into two groups. Troponin T and CPK-MB were measured preoperatively without drugs, after 12 to 15 days of drug/placebo administration,five minutes after aortic valve removal, and at 12, 24, and 48 hours follow-up. TnT and CPK-MB results reached significant differences (p=0.0001) in the treatment group compared with the control group at all four analysis periods. Echocardiographic examinations were also performed in the groups and showed no dynamics or differences between them. The authors concluded that pretreatment with trimetazidine reduced ischemia-reperfusion injury during CABG but showed no effect on left ventricular function.Lopatin and Dronova(2009) [109] added a series of 306 patients undergoing bypass surgery divided into two groups. Those taking trimetazidine (35 mg/MR per tablet) two weeks before surgery had significantly lower creatine kinase and creatine kinase MB levels at the 6th postoperative hour. Hei L et al. (2015) performed a systematic review and meta-analysis of six randomized controlled trials investigating the effectiveness of myocardial protection during preoperative trimetazidine therapy in CABG patients.

Postoperative creatine kinase (CK), creatine kinase MB(CK-MB), creatine phosphokinase (CPK), troponin T (TnT) and troponin I (TnI) concentrations were evaluated. Their results showed significantly lower postoperative levels of CK, CK-MB, TnT, and TnI in the trimetazidine-treated groups. The differences with our results can be commented as follows: all studies so far initiated trimetazidine therapy at least one week preoperatively;

the follow-up intervals were several times during the early postoperative period, when enzyme dynamics were greatest; in our study, we had no preoperative trimetazidine intake and the only serum sample in the early postoperative period in both groups was taken a few hours after the first administration of the drug. We can assume that at this time there is still no effect of trimetazidine that can be reported by CK-MB and TnT concentrations or that trimetazidine has no effect on isoenzyme MB and troponin T values in the first 6-8 hours after initiation of therapy with it. There was no TMZ intake in either group at baseline, so the lack of differences between groups is explainable. At the end point, there were also no statistical differences between the groups, but the values recorded were within the normal range. We can conclude that TMZ intake did not affect the baseline levels of the two biomarkers.

Regarding serum malondialdehyde levels, we observed differences in the two groups with statistical significance. The group of patients treated with trimetazidine had preoperatively higher mean MDA values, and the difference was statistically significant. During the second sampling at the 12th postoperative hour, serum MDA levels increased the most, equalizing in the two groups, with the difference in mean value not statistically significant. At the 1st postoperative month, we observed a decrease in serum malondialdehyde concentration, significantly more for the treatment group, and values below preoperative values were reached. For the control group, this decline was much less significant. At the 6th postoperative month, the trend of decreasing MDA levels remained in both groups, with the control group reaching preoperative serum concentrations and the treatment group reaching mean values well below preoperative.

Malondialdehyde was first used as a marker of oxidative stress levels in cardiac surgery patients taking trimetazidine, by Fabiani et al [99] in a randomized, double-blind, placebo-controlled study designed in 1992. The results showed that the increase in malondialdehyde in the coronary sinus 20 minutes after reperfusion was significantly reduced in the trimetazidine group. In the study by Vendrinne et al. (1996) [102], in patients on preoperative TMZ therapy, with no significant difference in the number of revascularized vessels, duration of aortic valve clamping, or cardiopulmonary bypass time, the authors noted slightly higher MDA levels in the treatment group. Interpretation of the study data showed no differences in FI between the two groups. Our results are similar to Iskensen et al. (2006) [108] who investigated the effect of preoperative TMZ use on reducing oxidative stress during CABG. The authors monitored serum superoxide dismutase (SOD), endogenous antioxidant enzyme systems of glutathione peroxidase (GPx) and malondialdehyde as markers of oxidative stress, in 24 patients divided into two groups. The results showed that postoperative antioxidant enzyme levels were significantly different between groups ($p = 0.05$), higher in the TMZ group, but MDA levels were significantly higher in the control group. There were no significant differences in haemodynamic parameters between the two groups. The difference with our study is that the patient cohort was significantly smaller and that TMZ administration was started preoperatively. The dynamics in MDA levels in the two groups we observed affected the total hospital stay, which was shorter for the treatment group, but did not affect the mean length of stay in intensive care. These differences are reflected in the correlations of the two groups versus MDA levels with mean times of ECC and aortic valve occlusion. In the control group, the duration of ECC and aortic valve clamping prominently affected MDA concentration. In the same group, the correlations were much more pronounced and over more time periods, in contrast to the treatment group, indicating that TMZ administration counteracted the increased oxidative stress levels from increased aortic valve and ECC duration. The amount of postoperative blood loss was not shown to have an effect on the reported malondialdehyde values.

The ejection fraction measured sonographically by Simpson's method showed no difference between the control group and the trimetazidine group. Similar results were found in the studies of Iskensen et al. Vendrinne et al. Martins et al.

In both groups in our study, there was a slight decrease in FI at the first hour after surgery, followed by an increase at discharge and at the 6th month, and the dynamics were the same for both groups. The only difference between the two groups with statistical significance was the inverse correlation between ejection fraction preoperatively and MDA levels at the 12th postoperative hour in the trimetazidine group, which we cannot interpret. We can conclude that postoperative administration of trimetazidine does not affect ejection fraction in coronary patients, despite reduced oxidative stress objectified by reduced malondialdehyde levels in the treatment group.

4. Tracking

Patients were followed up to the 6th month after the surgical intervention, according to indicators as explained in the **Materials and Methods** section. At their follow-up examinations, including up to the end of the first year, there was no recurrence of angina symptoms in any of the patients in either group.

5. Gender impact

There was no statistically significant difference by sex in mean MDA values, at all time points of the study for the entire study population (control and treatment group combined). Thus, the analyzed data showed no influence of gender on oxidative stress.

There was a significant difference in MDA levels between the control and treatment groups in both males and females at the 1st and 6th postoperative month, with values significantly higher in the control group. The dynamics were identical for both sexes.

When analyzing the data stratified by sex in the control and treatment groups, we also found a difference in MDA levels 12 hours before surgery in men, with values higher in the treatment group. We observed no such difference in women.

1. Influence of age

The mean age of the patients in the control group was 64.18 ± 6.54 years and for the treatment group was 62.97 ± 9.45 years. Most patients in both groups for both sexes were in the sixth decade, 61.2% of males and 43.5% of females, respectively. In dividing the patients into adult and non-elderly as per WHO definition, a cut-off of 68 years was adopted. Younger than this cutoff were 64 patients in the general population and 32 in the trimetazidine-treated group. We did not observe any differences in MDA levels when stratification was performed in this way in both the general population and the treatment group. We can conclude that age does not influence oxidative stress levels in coronary surgery.

2. Influence of left ventricular pump function

As a criterion for objectification of left ventricular function, we adopted the ejection fraction(EF) measured sonographically by Simpson's method. The echocardiographic examination for the study was performed 12 hours before surgery, at the 1st hour after leaving the operating room, at discharge and at the 6th month after surgery.

In both groups, there was a slight decrease in FI at the first hour after surgery (mean FI 44.69% preoperatively; 44.26% at the first hour), followed by an increase at discharge and at the 6th month, where the mean FI was 54.11%, with the same dynamics for both groups. There was no statistically significant difference between the two groups at all time points studied. There was a statistically significant inverse correlation between ejection fraction preoperatively and MDA levels at the 12th postoperative hour only in the trimetazidine group.

VI. Conclusions

1. The formed groups are comparable according to their initial characteristics; 2. Coronary surgery with extracorporeal circulation and clamping leads to increased levels of oxidative stress

3. Postoperative trimetazidine therapy reduces oxidative stress generated during coronary surgery;

4. Postoperative therapy with trimetazidine does not affect the functional capacity of the myocardium;

5. Reducing oxidative stress is not necessarily associated with improved biochemical and vital signs;

6. Taking trimetazidine in coronary patients may reduce the hospitalization period;

7. The sub-selections performed showed no influence of the factors studied on the reported trends;

VII. Scientific contributions in relation to the dissertation work

1. For the first time in Bulgaria, a thorough scientific study of the effect of trimetazidine on ischemia reperfusion injury in on pump coronary surgery has been performed based on an extensive literature review;

2. For the first time, the effects of trimetazidine administration entirely postoperatively in on pump coronary surgery have been reported;

3 .A special protocol for registration and postoperative follow-up was developed, as well as a system for measuring human malondialdehyde;

4. The efficacy of postoperative trimetazidine therapy on oxidative stress and myocardial functional capacity was evaluated;

5. The real benefits of postoperative administration of trimetazidine were evaluated;

IX. Reference to the "impact factor" of journals in which the submitted publications have been published

Total number of publications - 3 (realized), with impact factor - 1 (realized)