MEDICAL UNIVERSITY – PLEVEN

FACULTY OF MEDICINE
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COMORBIDITY, RISK PROFILE AND BIOMARKERS IN HOSPITALIZED PATIENTS WITH CHRONIC HEART FAILURE

Author's summary of dissertation for conferment the educational and scientific degree doctor of the scientific specialty cardiology

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CONTENTS

I. Introduction.....5

II. Literature review conclusions 6

III. Aims and tasks…….....7

1. Aims:…….....7
2. Tasks.....7

IV. Material and methods.....7

1.Material.....8
1.1. Clinical contingent- basic characteristics.....8

2.Methods.....9

V. Results…….....10

V. Discussion.....47

VI. Conclusions.....57

VIII. Contributions.....58

ABBREVIATIONS:

ACC American College of Cardiology
ACS acute coronary syndrome
нAHA American Heart Association
ACE I angiotensin converting enzyme inhibitor
AH arterial hypertension
AF atrial fibrillation
AMI acute myocardial infarction
BP blood pressure
BMI body mass index
BNP brain natriuretic peptide
CKF chronic kidney failure
COPD chronic obstructive pulmonary disease
CHF congestive heart failure
CI confidence interval
CAD coronary artery disease
CRP C-reactive protein
DM Diabetes mellitus
ECG electrocardiogram
ELISA enzyme-linked immunosorbent assay
eGFR estimated glomerular filtration rate
ESC European Society of Cardiology
ECCHF exacerbated chronic congestive heart failure
IL interleukin
IL-18BP interleukin18 binding protein
IL-18R-α interleukin -18 receptor alpha
IL-18R-*β*. interleukin -18 receptor beta
IHD ischemic heart disease
IVC inferior vena cava
GWGHF Get With Guidelines Heart Failure
HF Heart Failure
HFA Heart Failure Association
HFmEF heart failure with mid-range ejection fraction
HFpEF heart failure with preserved ejection fraction
HFrEF heart failure with reduced ejection fraction
LAHB left anterior hemiblock
LBBB left bundle branch block
LV left ventricle
mRNA matrix ribonucleic acid
NYHA New York Heart Association
NTproBNP N-terminal pro B-type Natriuretic Peptide
OR Odd ratio
PCWP pulmonic capillary wedge pressure
RBBB right bundle branch block
RF risk factor
SAP systolic arterial pressure
SCD sudden cardiac death
TAPSE tricuspid annular plane systolic excursion
TDI tissue dopler imaging
TNF-α Tumor necrosis factor alpha
VF ventricular fibrillation
VT ventricular tachycardia

I. INTRODUCTION

Heart failure (HF) is a syndrome which is a consequence from other disease: arterial hypertension (AH), ischemic heart disease (IHD), heart valve abnormality, tachyarrhythmias, bradyarrhythmias, cardiomyopathies (idiopathic, hypertrophic, restrictive), thyrotoxicosis, etc. There is growing evidence about IHD's role in that process because most of the patients are elderly, whereas heart valve abnormalities decrease their incidence. Noteworthy with increasing age, apoptosis has greater significance and the number of cardiomyocytes is decreased, therefore the heart's pump potential is limited favoring HF development. The aging of the population and longer life expectancy leads to increased cases of HF worldwide. According to the American Heart Association 6.2 million people in USA have HF betwen 2013-2016 and 23 million have HF worldwide. According to *Gottdiener J. S.* et al., congestive heart failure's (CHF) frequency increases with advancing age and it is higher in men than women.

Asymptomatic HF is told to be precursor of symptomatic HF and it is related to high mortality. The symptoms are very important but they must be confirmed from objective data of HF. The symptoms and the severity of cardiac dysfunction have mild association each other, but they have great significance in terms of prognosis, especially if they persist and they are at the base of classification of New York Heart Association (NYHA).

European society of cardiology (ESC) recommends differentiating patients with HF on the basis of left ventricular ejection fraction, because of different etiology, demographic characteristics, comorbidity and response to the applied therapy. In clinical practice patients with HF are often polymorbids, comorbidity is divided into two groups: non-cardiac and cardiac. *Chamberlain A. M.* et al., described that polymorbidity is common in patients with HF and most of these patients (86%) have two or more chronic conditions. That is why a more detailed study on the concomitant diseases is needed in both patient groups: heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF).

According to ESC guidelines of the therapy of HF from 2016 patients must be subjected to screening for cardiovascular and non-cardiovascular comorbidities. If such comorbidities are found, they must be managed in order to improve symptoms, general condition and the clinical outcome in the corresponding comorbidity without exacerbating heart failure. The increasing frequency and constantly high morbidity and mortality from CHF are assumed that risk factors (RF) remain unaffected by current treatment options for HF. Based on the current understandings in CHF the immune system is activated. This proves the role of inflammation in CHF. The inflammation is proposed to play a more complex role and it can also have an adaptive and cardioprotective effect.

ESC data shows that a number of prognostic markers of death and/or hospitalizations for HF are identified in patients with CHF, but their clinical application is limited and the exact risk stratification of HF remains a challenge. Several multifactor risk scores have been developed for different populations of HF patients in recent decades. Multifactor risk scores can help in the prognosis of death in patients with HF but remain less useful in predicting future HF hospitalizations.

II. LITERATURE REVIEW CONCLUSIONS

1. HF is a challenge with numerous RF leading to CHF, patients' polymorbidity, high mortality, deteriorating quality of life, lasting morphological changes, mechanisms of progression of HF including apoptosis and with relatively high treatment costs (about 2% of total healthcare costs).

2. More data is reported about etiopathogenesis, progression and prognosis of HF in recent decades. These data contribute to validation of a diagnostic algorithm and new treatment strategies of CHF.

3. Despite numerous studies on RF, unclarities in HF manifestation and prognosis exist.

4. A large number of population studies are being performed on cardiac and non-cardiac comorbidity in CHF but the independent meaning of some comorbidities as well the interaction between them in the HF manifestation and course has not been fully clarified.

5. Neurohumoral factors have not been studied in detail as mechanisms related with cytokine theory in the onset and development of HF. The prognostic significance of cytokines in HF has not been fully explored and biomarkers of early renal injury in the course of CHF are studied.

6. Based on the echocardiographic findings in CHF, the next groups of patients are defined: subjects with preserved left ventricular ejection fraction, with mid-range ejection fraction and with reduced left ventricular ejection fraction. These patients' RF concomitant diseases and prognosis are object of researches.

7. The role of proinflammatory factors in pathogenesis and progression of HF has not been fully studied. HF is a systemic disease with multifactorial etiology. A large number of studies show that activation of inflammation is a central factor in the onset and progression of HF. The immune system plays a key role in myocardial remodeling, hypertrophy, apoptosis and fibrosis. The balance between proinflammatory and anti-inflammatory cytokines is disturbed in HF. Further investigations are needed for evaluation of inflammatory mechanisms of HF. This would help in discovering of new opportunities for improving the quality of life, slowing the disease progression and improving patient survival.

8. The risk stratification for rehospitalization and death of patients with HF has not been fully revealed. In a systematic review investigating 64 prognostic models. *Rahimi K.* et al., processed data from Medline and EMBASE between 1995 - 2013 year. The researchers choose studies with at least one multivariate model for prediction of death, hospitalization or both. The autors analyse data from 64 models and conclude that several good validated models exist as predictors of mortality in HF, despite the fact that these models include few major risk markers. ESC reported meta-analysis and meta-regression study of 117 prognostic models which also show that multivariate models, for the assessment of risk of rehospitalizations and death in HF, are more informative in regards to mortality than rehospitalizations.

III. AIM AND TASKS

1. AIM

To study risk profile and the influence of comorbidity on the progression of heart failure in hospitalized patients with exacerbated chronic congestive heart failure (ECCHF) with preserved, mid-range and reduced left ventricular ejection fraction and assessment of three biomarkers**: natriuretic peptides, interleukin-18** as predictors of rehospitalizations and cardiovascular mortality in patients with HF and **Cystatin C** for specifying of renal injury in the course of HF.

2 .TASKS

2.1. To investigate demographic data, clinical manifestation and comorbidity in patients with HF and ECCHF hospitalized in University hospital "D-r Georgi Stranski" Pleven for the period of 2016-2018 and to evaluate concomitant diseases and their impact on rehospitalizations and progression of HF.

2.2. To analyze cardiac and non-cardiac comorbidity and its impact on the mortality in patients with HFrEF, HFmrEF and HFpEF (categories according to left ventricular ejection fraction assessment by echocardiography).

2.3. To estimate the predictive value of NT-proBNP and interleukin-18 for future unfavorable events in hospitalized patients with HF and examination of Cystatin C for specifying of early renal injury in the course of HF.

2.4. Prospective follow-up of the patients for two years in order to stratify the risk for rehospitalizations and cardiovascular mortality in patients with HF and formation of risk groups among the investigated subjects.

IV. MATERIAL AND METHODS

1. MATERIAL

1.1 .CLINICAL CONTINGENT – BASIC CHARACTERISTICS.

The current study is a prospective one.

The examined clinical contingent includes 337 patients hospitalized in Second clinic of cardiology in university hospital "D-r Georgi Stranski" Pleven for the period of 2016-2018. All patients are followed-up prospectively for 24 months. The scientific project was approved by the ethics commission of Medical University-Pleven. All participants were informed beforehand with the aims and methods of the study and they signed informed consent.

Study inclusion criteria: exacerbated chronic congestive heart failure (ECCHF) from II to IV F.C. NYHA. When we analyze HF progression we divided patients on subgroups with HFrEF, HFmrEF and HFpEF, as well patients' diastolic dysfunction according to the echocardiographic assessment of left ventricular EF and on subgroups according to patients' comorbidities.

Study exclusion criteria: known autoimmune disease, collagenoses, malignant disease, advanced renal disease (creatinine levels more than 250 μmol/l and hemodialysis), bacterial endocarditis, pulmonary thromboembolism, informed consent refusal. We registrated exclusion criterias in 9 from 337 patients, which reduced the examined contingent to 328. In 90 subjects was investigated a constellation from biomarkers (78 patients from the studied group and 12 controls).

DEMOGRAPHIC CHARACTERISTICS OF THE EXAMINED CONTINGENT

The examined contingent includes 328 patients were hospitalized in university hospital "D-r Georgi Stranski" Pleven for the period of 2016-2018 who are followed-up prospectively for a period of 24 months, 186 (56.7%) men and 142 (43.3%) women (fig. 1).

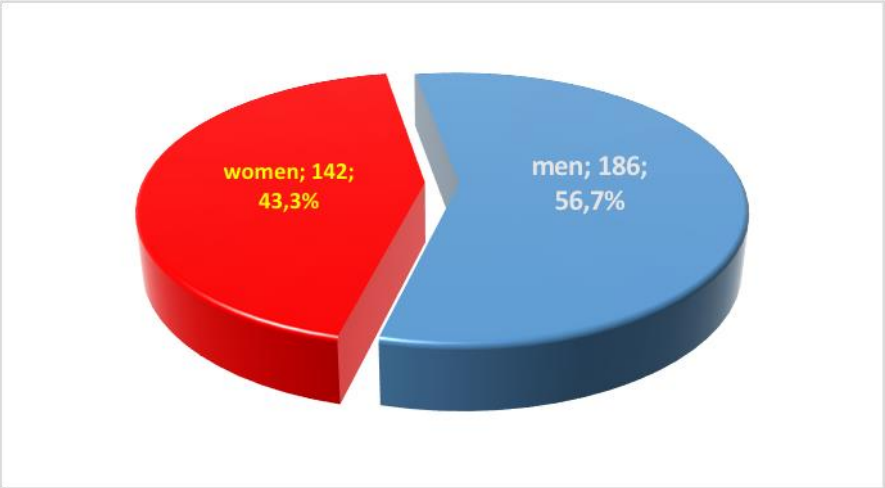


Figure 1: Contingent distribution by gender

The mean age of the study participants was 66.5 ± 10.31 years in the range of 30-89 years. In men the highest number (58) is the age group 60-69 years, followed by the group 70-79 with 56 male patients, and the least (2) are presented in the group 30-39 years. As for the women older patients predominate (50 in age group 70-79 years), followed by 47 in the group 60-69 years, whereas the smallest (2) are in the group 40-49 years. There were no women in the age group 30-39 years (fig. 2).

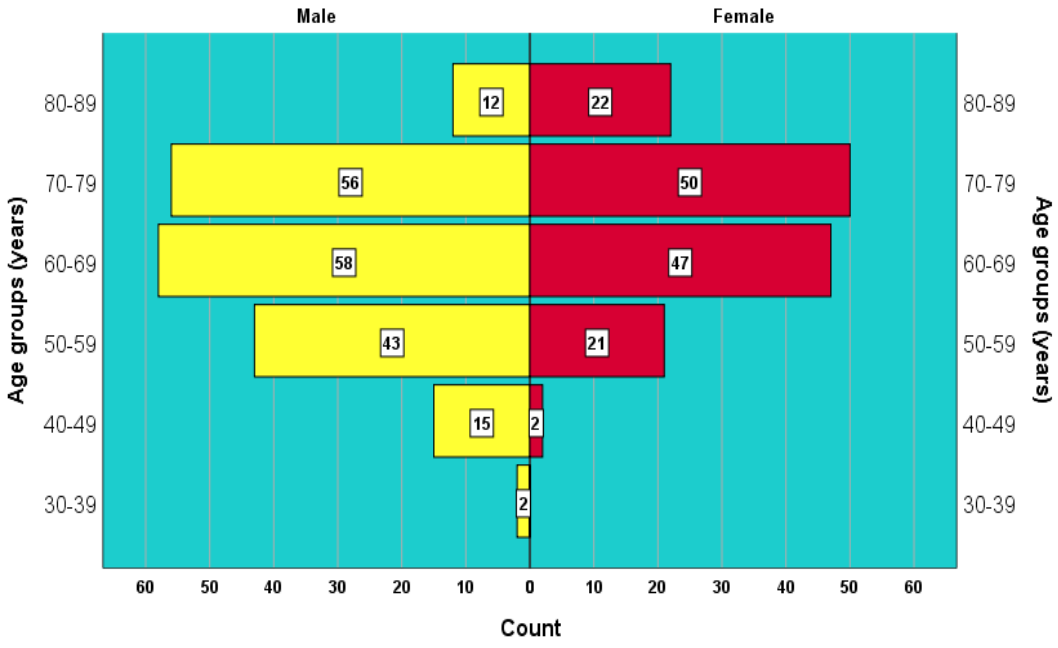


Figure 2: Contingent distribution by gender and age groups.

All patients with ECCHF included in the study were treated by standard protocol as: 94% have taken ACE-inhibitors and ARBs; 85% have taken beta-blockers; 96% were treated with diuretics; patients with atrial fibrillation have taken adequate anticoagulants therapy with vitamin K antagonists or non-vitamin K oral anticoagulants (NOAC). All patients have taken medicaments for treatment and control of the concomitant diseases

2. METHODS

2.1. Documentary method Patients' medical history RF, concomitant and past diseases, cardiac and non-cardiac comorbidity. Anamnesis data: smoking, overweight, diabetes mellitus (DM), AH, IHD, hyperuricemia, gout, anemia, chronic obstructive pulmonary disease (COPD).

2.2. Clinical method – Anamnesis, weight and height measurements, blood pressure (BP), pulse rate, and detailed physical examination.

2.3. INSTRUMENTAL TESTS:

2.3.1. Electrocardiography (ECG) - twelve leadings (with velocity 25 mm/sec and voltage 10 mm/mV)

2.3.2. Roentgenography of heart and lungs - for assessment of pulmonary congestion, configuration and heart borders.

2.3.3. Transthoracic echocardiography – for assessment of left ventricular (LV) ejection fraction (EF) and categorization of the patients according to the classification of European Cardiology Society 2016.

2.4. PREPARATION OF PATIENT'S QUESTIONNAIRE

A questionnaire was developed and all data of patients were prospectively filled as well all results from ECG, echocardiography, laboratory, the three studied biomarkers, data from the period of monitoring and the disease outcome.

2.5. Laboratory methods: examination of full blood count, blood glucose, creatinin levels, GFR, urea, electrolytes, lipid profile:

Glomerular filtration rate (GFR) is known to be the most accurate indicator of renal function. Normal GFR varies by age, gender, weight and decreases with advancing of age. GFR is calculated by using values of serum creatinine levels or Cystatin C, in combination with age, race and gender, applying the established formula (eGFR). GFR based on Cystatin C is more accurate indicator for renal function.

In the current study MDRDeGFR Calculator was used for serum creatinine levels in all patients and MDRDeGFR Calculator for Cystatin C in 78 patients.

- *GFR Calculator of NKF: www.kidney.org/gfr*

2.6. EXAMINATION OF BIOMARKERS (*in 78 patients with HF)*

All tests are performed in the laboratory of MDL Clinical Immunology, university hospital "D-r Georgi Stranski" Pleven, Second Clinical Base.

2.6. 1. NATRIURETIC PEPTIDES (NT-PROBNP)

The enzyme–linked immunosorbent essay (ELISA) was used for quantitative measurement of human NT-proBNP.

2.6.2. IL-18 Platinium ELISA was used for quantitative measurement of human IL-18.

2.6.3. CYSTATIN C was examined by turbodimetric method via automatic analisator SPAplus.

2.7. STATISTICAL METHODS

The research data was processed with the programs IBM SPSS statistics 25.0 and MedCalc Version 14.8.1. The level of significance which rejects the null hypothesis was determined as $p<0,05$. The following methods were applied:

2.7.1. Descriptive analysis – *distribution in tables of the examined features by groups.*
2.7.2. Variation analysis *– for assessment of the characteristics of the central tendency and statistical dispersion.*
2.7.3. χ2 – test and Fisher's test – *for search of relationship between categorical signs and hypothesis test for association between categorical variables. The level of significance which rejects the null hypothesis was determined as p<0,05.*
2.7.4. Comparison of relative proportions
2.7.5.. Graphical analysis – for visualization of the results
2.7.6. Non-parametrical test of Kolmogorov-Smirnov and Shapiro-Wilke for verification of normality distribution
2.7.7. ANOVA for comparison of mean arithmetic values of two independent factors
2.7.8. T criteria of Student – *for test hypothesis among two independent samples.*
2.7.9. Non-parametrical test of Kruskal-Wallis – *for comparison of more than two independent samples.*
2.7.10. Non-parametrical test of Mann-Whitney – *for test of hypothesis for difference between two independent samples.*
2.7.11. Correlation analysis for assessment of linear relationship between quantitative signs
2.7.12. ROC curve – *for estimation of threshold values of quantitative signs.*
2.7.13. Binary logistic regression – *for quantitative assessment of the examined factors and indicators*
2.7.14. Criteria for validation of screening tests - *the following criteria were used for validation of screening test: sensitivity; specificity; positive predicting value; negative predicting value; precision (% of true answers).*
2.7.15. Kaplan-Meier method *– for estimation of the time until onset of the examined event (Kaplan-Meier product limit estimation of the survival function).*

V. RESULTS

1. CLINICAL SYMPTOMS OF ECCHF DURING HOSPITALIZATION

All patients' symptoms associated with manifestations of HF during hospitalization were analyzed (fig.3): the highest proportion from clinical criteria for ECCHF is nocturnal dyspnea (56.4%), followed by dyspnea during mild physical activities (54.3%); and least cases with dyspnea during usual physical activities (4.9%).

Pleural effusions were observed as manifestation of decompensation of HF in 29 patients, in term of pericardial effusion and ascites: pleural effusion showed highest percentage (5.2%), followed by pericardial effusion with 2.4% and ascites – 1.2%.

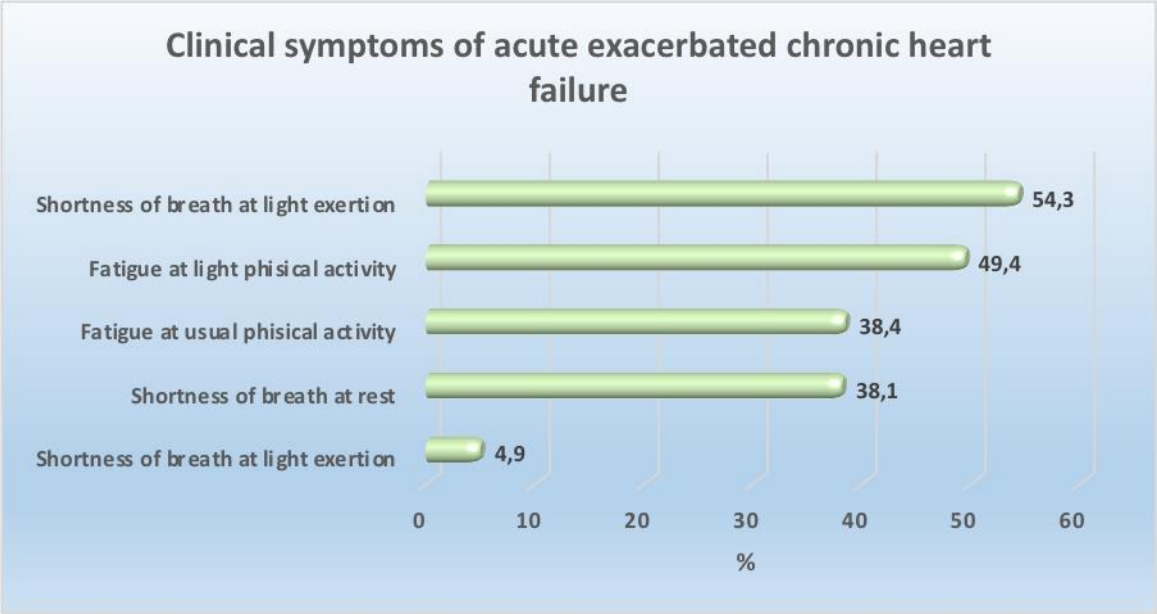


Figure 3: Distribution of the patients by clinical criteria for ECCHF (the sum of percentages exceeds 100, as some of the patients have more than one criterion).

2.BLOOD PRESSURE DURING HOSPITALIZATION

Patients with systolic arterial pressure (SAP) 120-140 mm/Hg predominate during hospitalization followed by patients with SAP > 140 mm/Hg. Patients with BP 120-140 mm/Hg during hospitalization showed highest proportion (35.1% BP), 31.1% showed 100-120 mm/Hg and the smallest number of cases showed SAP < 90 mm/Hg (0.6%).(fig.4).

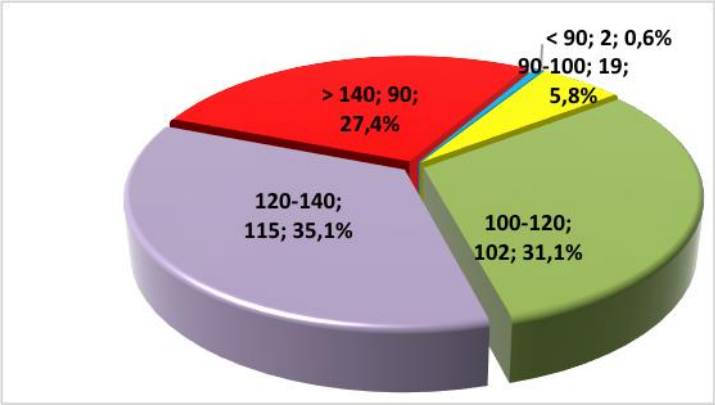


Figure 4: Distribution of the patients by blood pressure during hospitalization

3. COMORBIDITY

The present study followed-up and analyzed the concomitant diseases, course of the disease, complications, rehospitalizations and mortality for 24 months. Data from non-cardiac and cardiac comorbidity are described in table 1, 2, 3 and 4.

Non-cardiac comorbidity	N	%
COPD	83	25,3
Thyroid pathology	46	14,02
Diabetes mellitus	117	35,67
Duration of DM		
Newly diagnosed	14	11,95
Up to 5 years	40	34,20
From 5 to 10 years	42	35,90
Over 10 years	21	17,95
Anemia	135	41,16
CRF	87	26,5
Overweight	145	44,21
Gout	60	18,29

Table1. **Non-cardiac comorbidity**

The hospitalized patients with HF are often with more than one concomitant non-cardiac disease and patients with more two concomitant diseases predominate (25.3%), followed by these with one non-cardiac disease (24.7%). The proportion of patients with three comorbid diseases is also high. (table 2).

Table 2: Distribution of the patients by number of non-cardiac comorbid diseases

Number of non-cardiac comorbid diseases	n	%	Sp
0	41	12,5	1,8
1	81	24,7	2,4
2	**83**	**25,3**	2,4
3	76	23,2	2,3
4	31	9,5	1,6
5	16	4,9	1,2
Total	328	100,0	

The most common cardiac comorbidities in HF are: Ischemic heart disease (IHD), arterial hypertension (AH) and atrial fibrillation (AF) (table 3).

Cardiac comorbidity	N	%
AH	289	88,1
Duration of AH		
Up to 5 years	22	6,7
From 5 to 10 years	57	17,6
Over 10 years	210	66,0
Valve abnormality	191	58,23
IHD	272	82,92
CIHD ischemic cardiomyopathy	194	59,6
CIHD previous MI	62	19,6
ACS	16	5,1
AF before hospitalization	115	35,06

Table3. **Cardiac comorbidity**

The hospitalized patients with HF are polymorbid regarding cardiac pathology and they are often with more than two concomitant cardiac diseases. (table 4).

Table 4: Distribution of the patients by number of cardiac comorbid diseases

Number of cardiac comorbid diseases	N	%	Sp
0	2	0,6	0,4
1	31	9,5	1,6
2	74	22,6	2,3
3	**185**	**56,4**	2,7
4	36	11,0	1,7
Total	328	100,0	

Patients with three concomitant cardiac diseases show highest proportion (56.4%) followed by patients with two concomitant heart diseases (22.6%). Patients with one concomitant cardiac disease showed smallest number – 9.5% and only 0.6% were without.

4. FUNCTIONAL CLASS OF THE HOSPITALIZED PATIENTS

The distribution of the patients by NYHA functional class during hospitalization is presented on table 5. Patients with NYHA III F. C. show highest proportion (80.2%), followed by patients with II F. C. (16.8%) and IV F.C. (3.0%).

Table 5: Distribution of the patients by NYHA functional class (F.C.)

Functional class (F.C.) NYHA	Number	%	Sp
I	0	0,0	0,0
II	55	16,8	2,1
III	**263**	**80,2**	2,2
IV	10	3,0	0,9
Total	328	100,0	

5. **ARRHYTHMIAS**

87 patients were with arrhythmias or 26.5% from the study group (fig. 5), the most common arrhythmia is paroxysmal atrial fibrillation (12.5%) followed by ventricular extrasystoles – 11.6% and non-sustained ventricular tachycardia (VT) – 0,9% (table 6).

Table 6: Distribution of the patients by type of arrhythmia

Type of arrhythmia	Number	%	Sp
None	241	73,5	2,4
Paroxysmal supraventricular tachycardia	4	1,2	0,6
Ventricular extrasystoles	38	11,6	1,8
Non-sustained VT	3	0,9	0,5
Supraventricular extrasystoles	17	5,2	1,2
Paroxysmal atrial fibrillation	41	12,5	1,8

** - the sum of the percentages exceeds 100 because some of the patients have more than one type of arrhythmia*

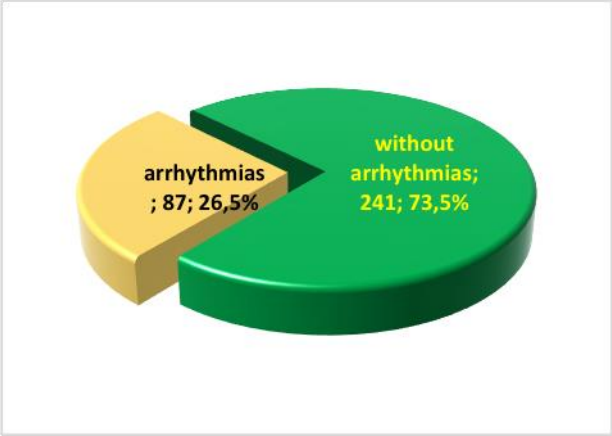


Fig. 5: Arrhythmias during hospitalization

6. **CONDUCTION ABNORMALITIES**

78 patients were with conduction abnormalities or 23.8% from the study group (fig. 8), the most common conduction abnormality was right ventricular bundle branch block (RBBB) – 9.5%; 7.9% were with left anterior hemi block (LAHB) and 6.1% were with left bundle branch block (LBBB) (table 7).

Table 7: Distribution of the patients by conduction abnormalities

Type of conduction abnormalities	Number	%	Sp
None	250	76,2	2,4
LAHB	26	7,9	1,5
LPHB	3	0,9	0,5
RBBB	31	9,5	1,6
LBBB	20	6,1	1,3
1st degree AV block	10	3,0	0,9
2nd degree AV block	1	0,3	0,3
3rd degree AV block	3	0,9	0,5
Sinus bradycardia	1	0,3	0,3

** - the sum of the percentages exceeds 100 because some of the patients have more than one type of arrhythmia*

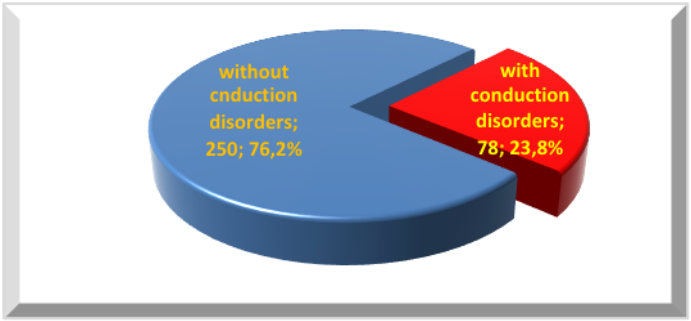


Fig.6. Distribution of the patients by type of conduction abnormalities

7. LEFT VENTRICULAR HYPERTROPHY

Approximately 72% from the studied participants had left ventricular (LV) hypertrophy (table 8, figure 7).

Table 8: – Analysis of the relation between LV hypertrophy and AH duration

Left ventricular hypertrophy	Number	%	Sp
No	92	28,0	2,5
Yes	236	72,0	2,5
Total	328	100,0	

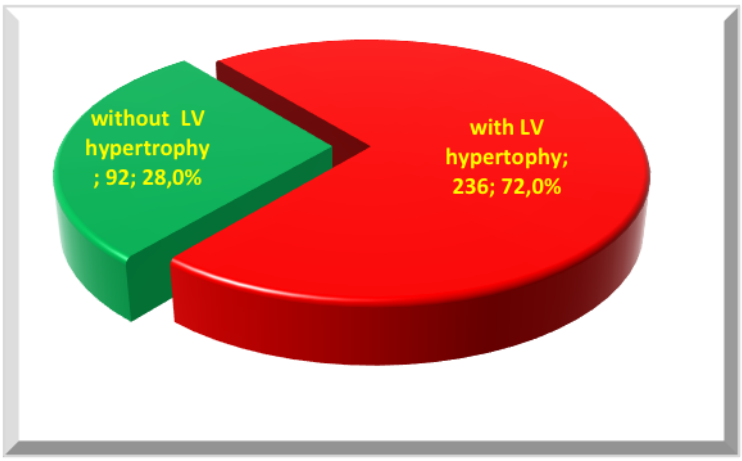


Figure 7: Distribution of the patients by left ventricular hypertrophy

There is no significant relation between LV hypertrophy and AH duration (table 9).

Table 9. Duration of AH and left ventricular hypertrophy

Duration of AH	LV hypertrophy				P
	No		Yes		
	N	%	N	%	
No	12	13,0	21	8,9	0,446
Up to 5 years	7	7,6	15	6,4	
From 5 to 10 years	13	14,1	48	20,3	
Over 10 years	60	65,2	152	64,4	

8. LEFT VENTRICULAR EJECTION FRACTION AND FUNCTIONAL CLASS

Since all patients with diastolic dysfunction have also preserved EF ≥ 50% but not all patients with preserved EF ≥ 50% have diastolic dysfunction we differentiate 4 patients categories: with preserved EF ≥ 50%; with preserved EF ≥ 50% + diastolic dysfunction; with mid-range EF - 49% and reduced EF < 40%.

Table.10 Distribution of the patients by left ventricular ejection fraction

Left ventricular ejection fraction	Number	%	Sp
With preserved EF ≥ 50%	205	62,5	2,7
With mid-range EF 40 – 49%	88	26,8	2,4
With reduced EF < 40%	35	10,7	1,7
Diastolic dysfunction	110	33,5	2,6

** - the sum of the percentages exceeds 100 because some of the patients with preserved EF have diastolic dysfunction*

Patients with preserved EF ≥ 50% showed highest percentage (62.5%), followed by patients with diastolic dysfunction – 33.5%. Patients with reduced EF < 40% were the smallest percentage from the cases (10.7%) (table 10, figure 8).

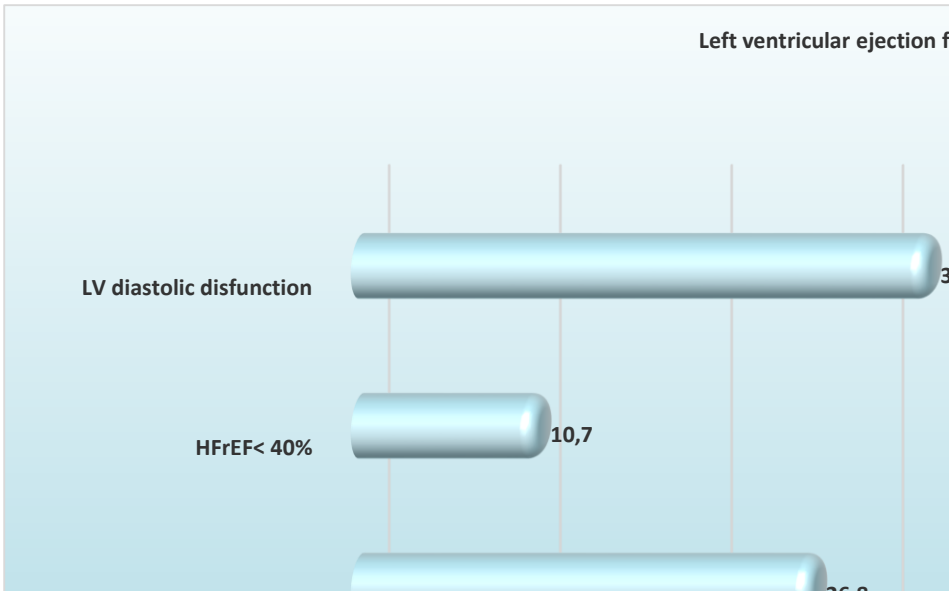


Figure 8: Distribution of the patients by left ventricular ejection fraction
(the sum of percentages exceeds 100 because some of the patients with preserved EF have diastolic dysfunction)

Patients with reduced EF < 40% showed significant difference in distribution of NYHA F. C. in the different categories of LV EF – the proportion of II F. C. is significantly lower than these with III and IV F. C. (the last two F. C. did not differ with each other) (table 11).

Table 11: Analysis of relation between LV EF and NYHA F.C.

Left ventricular ejection fraction	**Functional class (F.C.) NYHA**					
	II		III		IV	
	N	%	n	%	N	%
With preserved EF ≥ 50%	21	38,2[a]	73	27,8[a]	1	10,0[a]
With mid-range EF 40 – 49%	12	21,8[a]	73	27,8[a]	3	30,0[a]
With reduced EF < 40%	1	1,8[a]	32	12,2[b]	2	20,0[b]
With preserved EF ≥ 50% + diastolic dysfunction	21	38,2[a]	85	32,3[a]	4	40,0[a]

** - the same letters horizontally mean the absence of a significant difference, and the different ones - the presence of significant difference (p <0.05).*

Patients with NYHA III F.C. showed highest proportion among the four examined EF categories, followed by II F.C. in most of the cases (with the exception of patients with reduced EF and on last place IV F.C.) (fig.9).

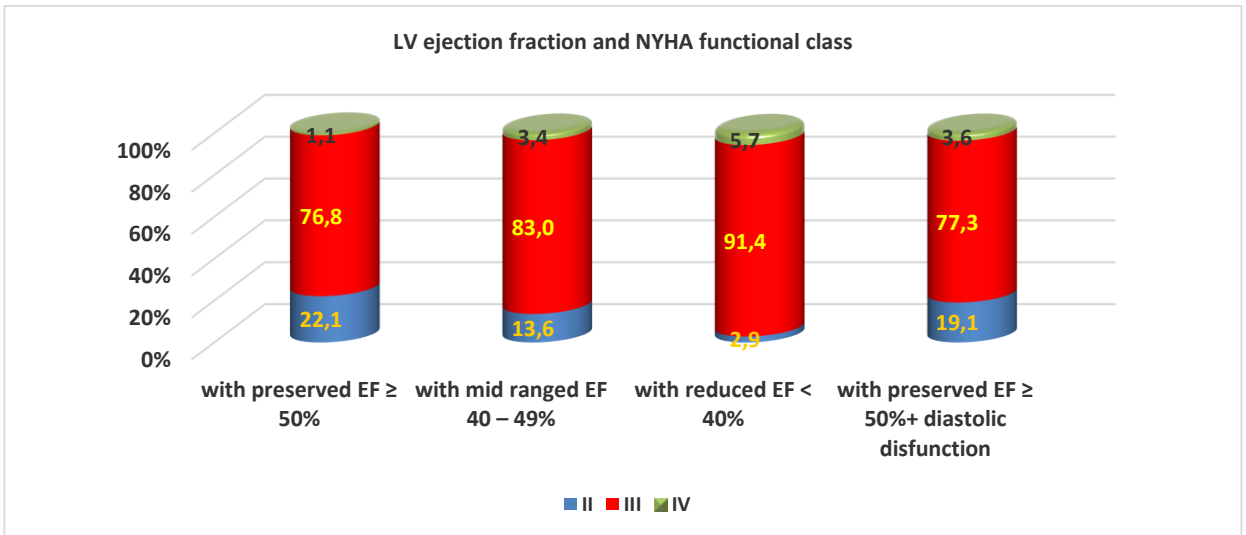


Figure 9: Distribution of the patients by LV EF and NYHA F.C.

9. BIOMARKERS

Three biomarkers were studied in 78 of the patients included in our study:

- NT-pro BNP – as "gold standard" in diagnosis and prognosis of HF;
- IL-18 – as biomarker of extracellular activation and inflammation, as a biomarker for the main pathogenic mechanisms of the onset and progression of HF (neurohormonal activation, myocardial stress, extracellular remodeling, comorbidity). IL-18 correlates with EF, its concentration increases with the progression of HF, it is also associated with ischemic renal injury (RI);
- Functional markers of RI were also examined – assessment of the degree of glomerular filtration via creatinine and Cystatin C levels.
- Cystatin C – is a marker for detection of early RI; its prognostic value is increased in combination with BNP
- eGFR-$_{MDRD}$ Cystatin C and eGFR$_{-MDRD}$ creatinine were calculated.

Tables 12, 13 and figures 10, 11 demonstrate that Cystatin C significantly correlates with creatinine and eGFR-creatinine; as for creatinine the correlation is directly proportional while with eGFR-creatinine the correlation is multidirectional.

Table 12: Correlation coefficients between creatinine, eGFR-creatinine and Cystatin C
****** - p<0,001***

Indicators	Cystatin C
Creatinine	0,514***
eGFR Creatinine	-0,604***

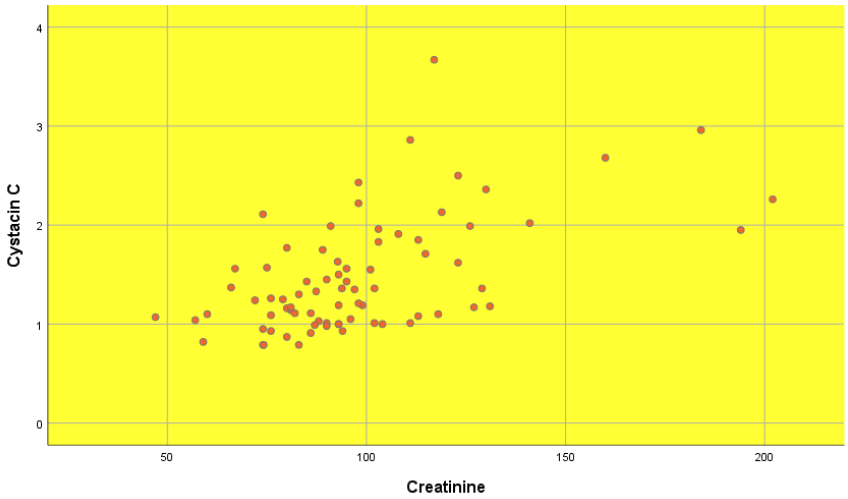


Figure 10: diagram (Scatterplot) of dispersion between creatinine and Cystatin C

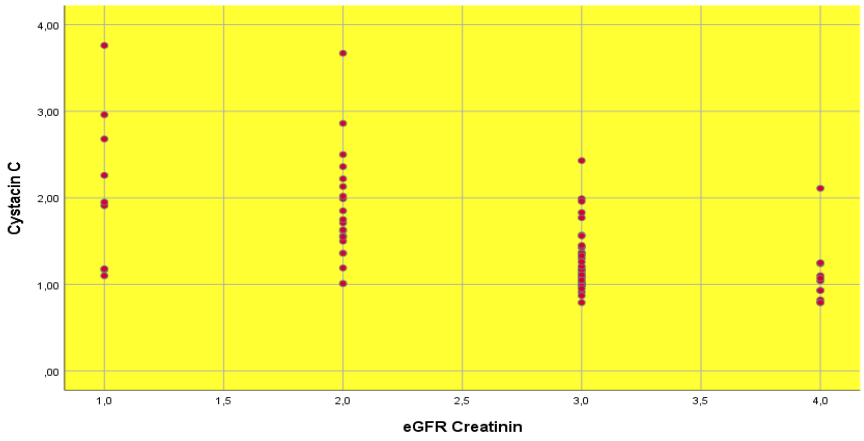


Figure 11: diagram of dispersion between eGFR-creatinine and Cystatin C

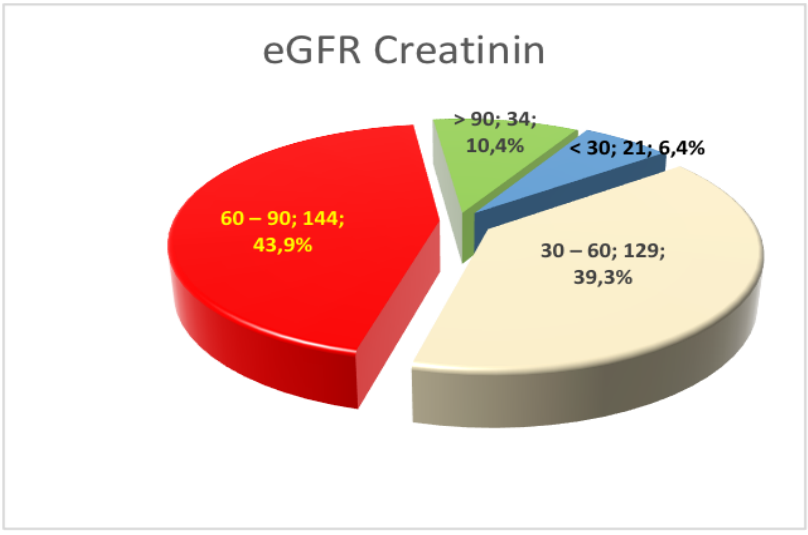
- **Patients with eGFR-Cystatin C between 60 and 90 ml/min/1,73 m2 are with significantly higher percentage of normal creatinine**.

Table 13: Analysis of relation between eGFR-creatinine and eGFR-Cystatin C values

eGFR Creatinine (ml/min/1,73 m^2)	Frequency	eGFR Cystatin C (ml/min/1,73 m^2)			
		< 30	30 – 60	60 – 90	> 90
< 30	N	4	5	0	0
	%	26,7[a]	14,7[ac]	0,0[bc]	0,0[ac]
30 – 60	N	8	11	2	0
	%	53,3[a]	32,4[ac]	8,7[bc]	0,0[ac]
60 – 90	N	2	16	16	3
	%	13,3[a]	47,1[ac]	69,6[bc]	50,0[ac]
> 90	N	1	2	5	3
	%	6,7[a]	5,9[a]	21,7[a]	50,0[a]

* - *the same letters horizontally mean no significant difference, while the different - presence of such (p <0,05)*

•In term of eGFR-creatinine (ml/min/1,73 m^2) (fig. 12): patients' values in interval 60-90 showed highest percentage (43.9%), followed by these with 30-60 (39.3%) and the smallest number of cases (6.4%) showed values under 30.



Фигура 12: Distribution of the patients by eGFR-creatinine

eGFR was used as more accurate criteria for analyzing of renal function. eGFR-creatinine and eGFR-Cystatin C were calculated and results were compared. We accepted as criteria for mild renal injury eGFR < 90 and ≥ 60 ml/min 1,73 m2, moderate – eGFR > 30 - < 60 ml/min 1,73 m2 and advanced kidney disease by eGFR < 30 ml/min/1,73 m2.

- Figure 13 shows that **89.7% from the studied participants are with increased levels of Cystatin C.**

In term of **eGFR-Cystatin C (ml/min 1,73 m2)** (fig. 14):

-the highest proportion of patients (43.6%) showed values in the interval 30-60, followed by these

- with 60-90 (29.5%)

- and least number of cases with **eGFR** higher than 90 – 7.7%.

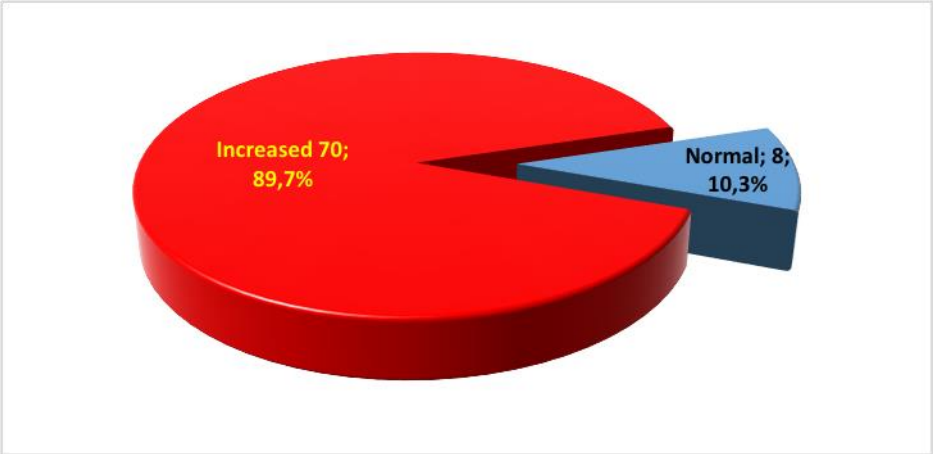


Figure 13: Distribution of the patients by Cystatin C

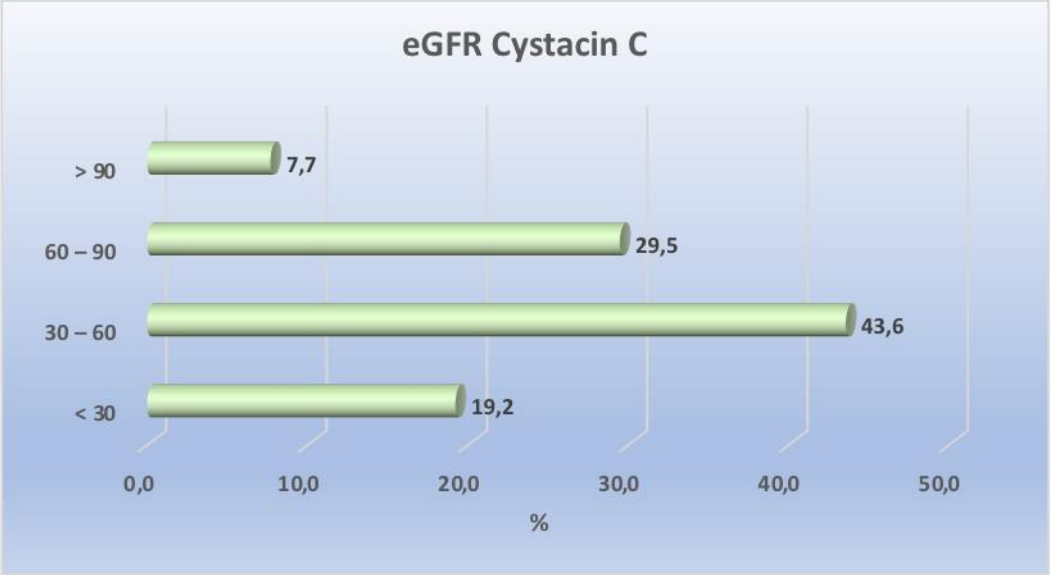


Figure 14: Distribution of the patients by eGFR-Cystatin C

Creatinine clearance is not a fully accurate indicator for assessment of glomerular filtration. That is why the examination of Cystatin C has been proposed, which has a number of advantages of creatinine clearance. Serum Cystatin C is an endogenous proteinase inhibitor that is synthesized by all nuclear cells. Cystatin C is a better marker than serum creatinine for detecting early kidney disease. It has been defined as an “ideal kidney marker” because it is

not affected by factors such as age, gender, muscle mass and is therefore suitable for use in children and adults with reduced muscle mass. In addition, Cystatin C is not dependent by inflammatory processes and it has an extremely low 24 h variation compared to the proven 40% variation for creatinine and it is also increased in minimal renal injury, contrary to creatinine which changes significantly only when 50% of renal nephrons are injured.

Tables 14, 15 and figures 16, 17 reveal that: there **is statistically significant relationship between NT-proBNP, Cystatin C and eGFR-Cystatin C values.** The correlation of eGFR-Cystatin C with the considered biomarkers is inversely proportional, strong with NT-proBNP and very strong with Cystatin C (tables 14, 15).

The relationship type with NT-proBNP is linear (fig. 16) whereas with Cystatin C – nonlinear (fig. 17).

Table 14: Analysis of the relationship between the biomarkers NT-proBNP, Cystatin C and eGFR-Cystatin C values

Biomarkers	Frequency	eGFR Cystatin C (ml/min/1,73 m^2)			
		< 30	30 – 60	60 – 90	> 90
NT-pro BNP					
Normal	N	1	5	13	4
	%	6,7[a]	14,7[a]	56,5[b]	66,7[b]
Increased	N	14	29	10	2
	%	93,3[a]	85,3[a]	43,5[b]	33,3[b]
Cystatin C					
Normal	N	0	0	2	6
	%	0,0[a]	0,0[a]	8,7[a]	100,0[b]
Increased	N	15	34	21	0
	%	100,0[a]	100,0[a]	91,3[a]	0,0[b]

** - the same letters horizontally mean no significant difference, while the different - presence of such (p<0,05)*

Table 15: Correlation coefficients between biomarkers NT-proBNP, Cystin C and eGFR-Cystatin C

Biomarkers	eGFR Cystatin C (ml/min/1,73 m^2)
NT-pro BNP	-0,613***
Cystatin C	-0,993***

****** - p<0,001***

In the population of subjects with higher values of NT-proBNP (sign of advanced HF), patients with eGFR-Cystatin C > 30 - < 60 ml/min 1,73 m2 and eGFR-Cystatin C ≥ 60 - < 90 ml/min/1,73 m2 is statistically significantly higher which confirms the thesis that when HF advances, early renal injury occurs. This is predisposition for development of cardiorenal syndrome. Our results also confirm the thesis that eGFR-Cystatin C is a more accurate marker for assessment of the renal injury stage, as well from the recent literature data which report that predictive value of Cystatin C and eGFR-Cystatin C increases in parallel measurement NT-proBNP.

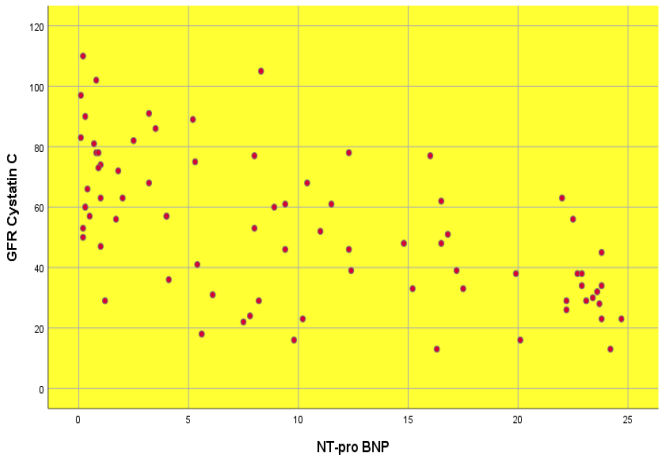


Figure 15: Diagram of dispersion between NT-proBMP and eGFR-Cystatin C

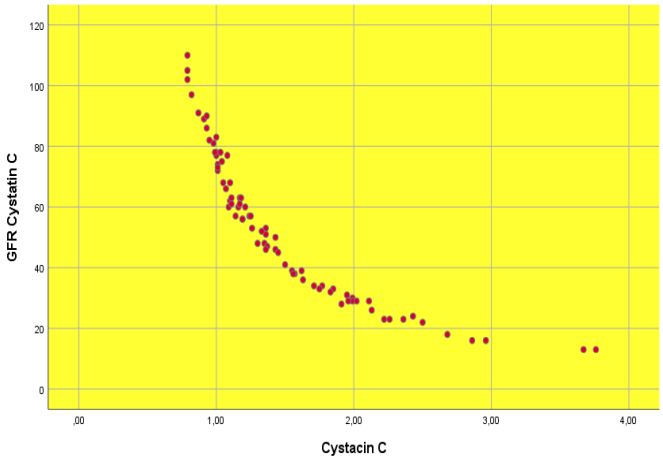


Figure 16: Diagram of dispersion between Cystatin C and eGFR-Cystatin C

Tables 16 and 17 show that there is no significant association between NT-proBNP, IL-18 values and the indicators LV EF and NYHA F.C.

Table 16: Analysis of the relationship between biomarkers NT-proBNP, IL-18 and the indicators LV EF and NYHA F.C.

Indicators	**NT-pro BNP**					**IL-18**				
	Normal		Increased		P	Normal		Increased		P
	n	%	n	%		N	%	n	%	
LV EF										
With preserved EF ≥ 50%	3	13,0	8	14,5	0,854	7	17,5	4	10,5	0,574
With mid-range EF 40 – 49%	11	47,8	22	40,0	0,701	13	32,5	20	52,6	0,117
With reduced EF < 40%	2	8,7	8	14,5	0,743	4	10,0	6	15,8	0,669
With preserved EF ≥ 50% + Diastolic dysfunction	7	30,4	17	30,9	0,822	16	40,0	8	21,1	0,118
NYHA F.C.										
II	6	26,1	9	16,4	0,499					
III	15	65,2	42	76,4	0,466					
IV	2	8,7	4	7,3	0,800					

Table17: Analysis of the relationship between quantitative values of NT-proBNP, IL-18 and the indicators LV EF and NYHA F.C.

Indicators	Biomarkers					
	NT-pro BNP			IL-18		
	N	$\overline{X}$	SD	n	$\overline{X}$	SD
LV EF						
With preserved EF ≥ 50%	11	8,34[a]	8,14	11	171,30[a]	244,55
With mid-range EF 40 – 49%	33	10,56[a]	8,96	33	157,92[a]	114,91
With reduced EF < 40%	10	12,94[a]	8,40	10	178,86[a]	122,66
With preserved EF ≥ 50% + Diastolic dysfunction	24	9,28[a]	8,59	24	132,89[a]	129,56
NYHA F.C.						
II	15	7,13[a]	7,95			
III	57	11,26[a]	8,85			
IV	6	7,25	5,99			

* - *the same letters vertically mean no significant difference, while the different - presence of such (p<0,05)*

** - *categories with a number of cases below 8 do not participate in the analysis due to lack of statistical representation*

10. REHOSPITALIZATIONS AND MORTALITY OF THE EXAMINED CONTINGENT

10.1. REHOSPITALIZATIONS

The total number of rehospitalizations is 97 – 62 (63.9%) were because of HF deterioration, 35 (36.1%) because of other reasons (fig. 17).

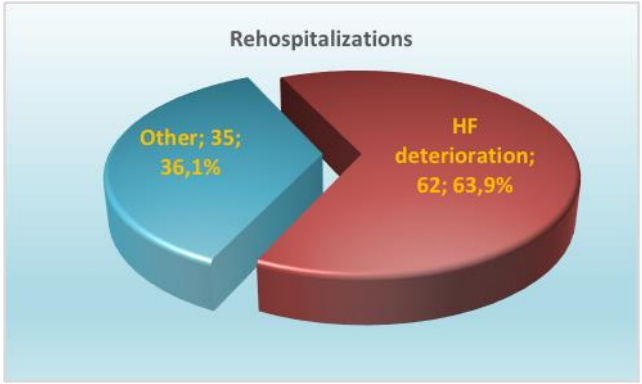


Figure 17: Distribution of rehospitalizations (3rd – 24th month) by reasons

By the end of the third month there were 12 rehospitalizations and 9 of the patients were rehospitalized for cardiovascular (CVD) reasons: 6 – because of ECCHF one – hospitalized for acute coronary syndrome and one patient with paroxysm of atrial fibrillation. From the three patients who were rehospitalized for other (non-CVD) reasons: one was hospitalized for chronic renal failure (CRF); one had trigeminal neuralgia and one is hospitalized in Clinic of Endocrinology for decompensated diabetes mellitus.

There are 23 rehospitalizations because of CVD reason until sixth month, 19 for ECCHF, 2 for acute myocardial infarction (AMI) and 1 because of ischemic stroke (IBI) and 1 hospitalization for infectious endocarditis. 13 hospitalizations for non-cardiovascular reasons were observed during the six month follow-up period: cholelithiasis, inguinal hernia, bronchopneumonia, influenza, 3-exarcebation of COPD, 3-decompensated diabetes mellitus, discopathy, right lower leg fracture with pulmonary thromboembolism (PTE), arthrosis with superimposed inflammatory process.

The rehospitalizations because of CVD reasons by the end of the first year were 28 – ECCHF 22, IBI – 2, ACS – 2 and 1 – because of implantation of permanent pacemaker for complete AV-block; hospitalizations for other diseases are: exacerbated chronic respiratory failure – 1, gout arthritis – 1, exacerbated COPD – 2, duodenal ulcer – 1.

13 patients were rehospitalized for CVD reasons – ECCHF, by the end of the second year and 3 patients were hospitalized because of other reasons: 1 – breast cancer, 1 – anemia and 1 because of acute gastrointestinal bleeding in the course of therapy with non-vitamin K oral anticoagulant (NOAC) for permanent atrial fibrillation.

Most rehospitalizations (36) occurred in the sixth month followed-up, and the least (12) – in the third month. In the all four follow-up periods the predominating reasons for rehospitalizations were deteriorating of HF. Its percentage was highest (81.3) in rehospitalizations at 24th month and lowest in the rehospitalizations at the sixth month – 52.8% (table 18) (fig. 18).

Table 18: Distribution of rehospitalizations by period and causes

Causes for rehospitalization	**Rehospitalizations**							
	3rd month		6th month		12th month		24th month	
	n	%	N	%	N	%	N	%
Deterioration of HF	8	66,7	19	52,8	22	66,7	13	81,3
Other	4	33,3	17	47,2	11	33,3	3	18,8
Total	12		36		33		16	

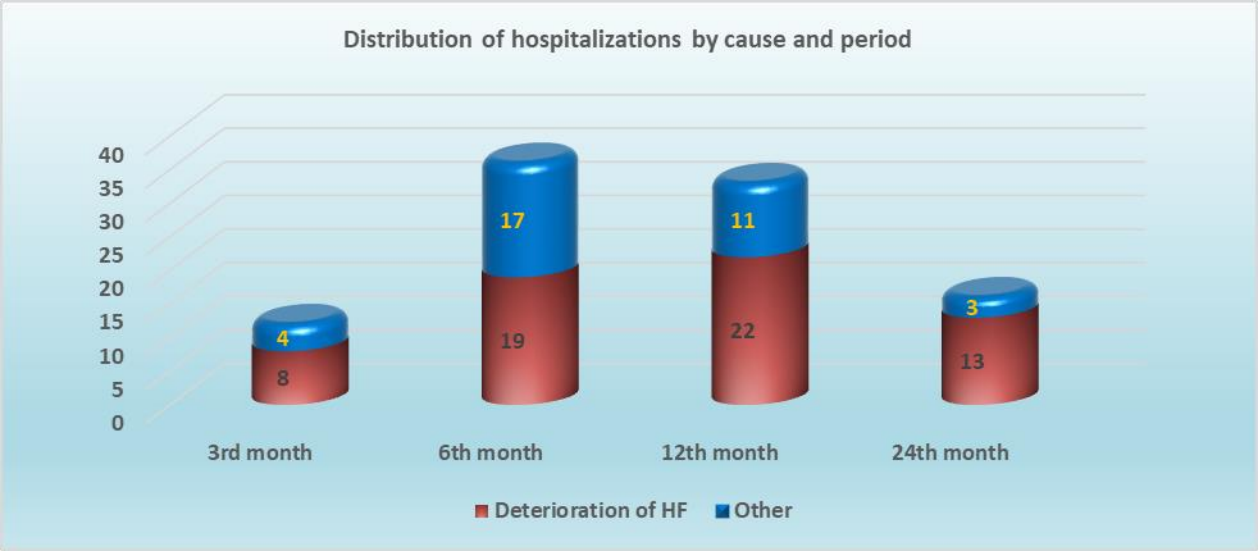


Figure18: Distribution of rehospitalizations by period and causes

10.2. MORTALITY

The total number of deaths was 45 or 13.7%. 28 (62.2%) were men and 17 (37.8%) – women. The proportion of deaths because of CVD reasons is significantly higher – 40 (88.9%) vs. deaths from other reasons – 5 (11.1%) (fig.19).

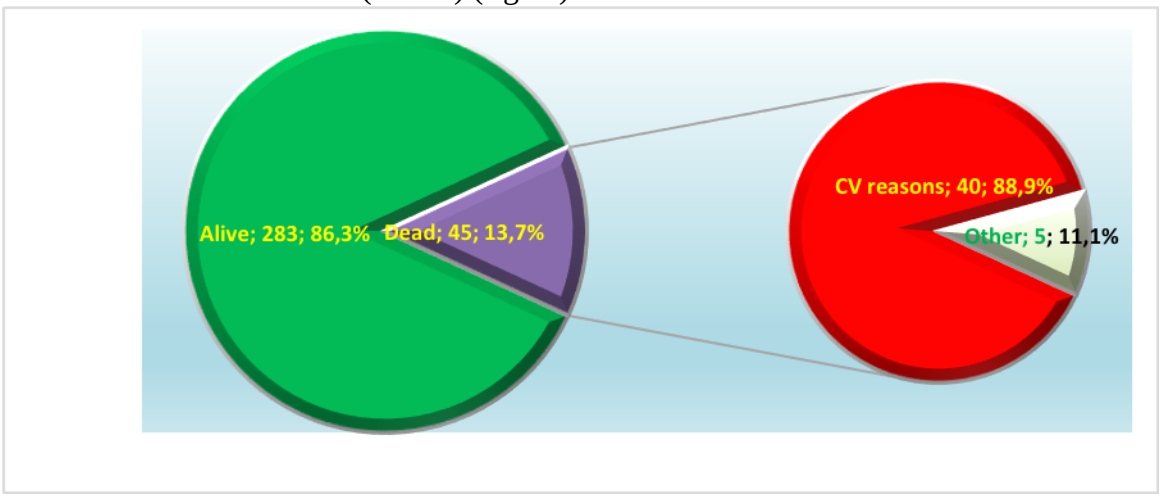


Figure 19: Total mortality distribution and mortality by causes

- **CAUSES OF DEATHS DATA**

During the prospective follow-up we analyzed the total and cardiovascular mortality. The most common cause of death in patients with HF is sudden cardiac deaths (SCD) in 17 of the patients (42.5%), followed by refractory CHF with contractile failure – 11 patients (27.5%), cardiogenic shock – 7 (17.05%), AMI – 4 (10.0%), chronic respiratory failure as a complication in patients with total HF – 3 (7.5%), IBI – 2 (5%).

The non-cardiac death causes in the current study were: CKD in two patients, malignancy in one, acquired coagulation factors deficiency in one patient with mitral valve's prosthesis, gastrointestinal bleeding – 1 (because of permanent AF treated with NOAC).

11. ANALYSIS OF THE RELATIONSHIP BETWEEN COMORBIDITY, REHOSPITALIZATIONS AND MORTALITY IN HF

The frequency of rehospitalizations and mortality in the studied patients for three-, six-, twelve-, and twenty-four months period and the influence of cardiac and non-cardiac comorbidity were analyzed. Practically there are no rehospitalizations until 30 days of HF. Data of rehospitalizations because of HF deterioration at the time of assessment on 3rd, 6th, 12th and 24th months were used. We did not find a significant relationship between any of the non-cardiac and cardiac comorbidities and rehospitalizations included in the study because of HF deterioration at 3rd, 6th, 12th and 24th months.

We found that **the increase in the number of cardiac comorbidities in the studied contingent is associated with higher rate of rehospitalizations because of HF deterioration, while in non-cardiac comorbidities this trend is not clearly expressed**; the only significant difference in the relative proportions of the non- and rehospitalized subjects was established in the rehospitalizations for HF at 24th month (table 19). The percentage of non-rehospitalized subjects from the patients without cardiac comorbidity was significantly higher **(p=0,039).**

Table 19: Analysis of the relationship between number of non-cardiac and cardiac comorbidities and rehospitalizations for HF at 24th month

Number of non-cardiac and cardiac comorbidities	Rehospitalizations for HF at 24th month				P
	No		Yes		
	n	%	n	%	
Non-cardiac					
0	35	12,3	1	7,7	0,950
1	70	24,6	4	30,8	0,859
2	76	26,8	4	30,8	0,999
3+	103	36,3	4	30,8	0,914
Cardiac					
0	1	0,4	0	0,0	**0,039**
1	30	10,6	0	0,0	0,442
2	63	22,2	3	23,1	0,791
3+	190	66,9	10	76,9	0,653

We did not find a significant relationship between any of the non-cardiac and cardiac comorbidities and mortality from HF at 6th month. The analysis of data from table 20 and 21 showed:

- **Significant relationship between mortality from HF at 12th month with duration of DM, gout and IHD;**
- The relative proportion of the dead patients in the period of one year is significantly higher than those of survived in the same period **with diabetes duration between 5 and 10 years (p=0,011);**
- The relative proportion of the dead patients with **gout** is **significantly higher than subjects without gout (p=0,013);**
- Patients with **chronic IHD with previous MI showed significantly higher percentage of deaths (p=0,039).**

The results of table 22 show a statistically significant relationship of mortality from HF until 24th month only with:

- **The cardiac comorbidity AF prior hospitalization** – the percentage of dead patients is significantly higher than the survived one in this period of follow-up **(p=0,003).**

Table 20: Analysis of the relationship between non-cardiac comorbidity and mortality from HF at 12th month

Non-cardiac comorbidity	Mortality from HF at 12th month				P
	No		Yes		
	n	%	n	%	
COPD					1,000
No	223	74,3	9	75,0	
Yes	77	25,7	3	25,0	
Thyroid pathology					1,000
No	261	87,0	11	91,7	
Yes	39	13,0	1	8,3	
Diabetes mellitus					0,131
No	193	64,3	5	41,7	
Yes	107	35,7	7	58,3	
Duration of DM					
Newly diagnosed	15	5,0	0	0,0	0,916
Up to 5 years	37	12,3	2	16,7	0,994
From 5 to 10 years	36	12,0	5	41,7	**0,011**
Over 10 years	19	6,3	0	0,0	0,779
Anemia					1,000
No	178	59,3	7	58,3	
Yes	122	40,7	5	41,7	
CRF					1,000
No	218	72,7	9	75,0	
Yes	82	27,3	3	25,0	
Obesity					0,773
No	166	55,3	6	50,0	
Yes	134	44,7	6	50,0	
Gout					**0,013**
No	247	82,3	6	50,0	
Yes	53	17,7	6	50,0	

Table 21: Analysis of the relationship between cardiac comorbidity and mortality from HF at 12th month.

Cardiac comorbidity	Mortality from HF at 12th month				P
	No		Yes		
	n	%	n	%	
AH					1,000
No	29	9,7	1	8,3	
Yes	271	90,3	11	91,7	
Duration of AH					
Up to 5 years	20	6,7	1	8,3	0,712
From 5 to 10 years	53	17,7	3	25,0	0,793
Over 10 years	198	66,0	7	58,3	0,810
Valve abnormality					0,132
No	126	42,0	2	16,7	
Yes	174	58,0	10	83,3	
IHD					
No	50	16,7	0	0,0	0,252
CIHD ischemic cardiomyopathy	180	60,0	5	41,7	0,334
CIHD previous MI	55	18,3	6	50,0	**0,019**
ACS	15	5,0	1	8,3	0,874
AF before hospitalization					0,063
No	192	64,0	11	91,7	
Yes	108	36,0	1	8,3	

Table 22: Analysis of the relationship between cardiac comorbidity and mortality from HF at 24th month.

Cardiac comorbidity	Mortality from HF at 24th month				P
	No		Yes		
	n	%	n	%	
AH					0,679
No	28	9,8	2	11,8	
Yes	259	90,2	15	88,2	
Duration of AH					
Up to 5 years	19	6,6	1	5,9	0,696
From 5 to 10 years	51	17,8	1	5,9	0,350
Over 10 years	189	65,9	13	76,5	0,525
Valve abnormality					0,801
No	119	41,5	8	47,1	
Yes	168	58,5	9	52,9	
IHD					
No	47	16,4	3	17,6	0,836
CIHD ischemic cardiomyopathy	172	59,9	11	64,7	0,890
CIHD previous MI	53	18,5	3	17,6	0,819
ACS	15	5,2	0	0,0	0,699
AF before hospitalization					**0,003**
No	190	66,2	5	29,4	
Yes	97	33,8	12	70,6	

To analyze the relationship between the number of non-cardiac and cardiac comorbidities and mortality at 6th, 12th and 24th month, patients were divided into 3 groups:

- Without disease;
- With one disease;
- More than one disease..

The results demonstrate that there is no significant relationship between number of non-cardiac and cardiac comorbidities and mortality from HF at 6th month (table 23), at 12th and 24th month (table 24, 25);

The proportion of survived patients until 12th month without cardiac comorbidity is significantly higher (p=0,011) and at 24th month (p=0,034) as well (table 24, 25).

Table 23: Analysis of the relationship between number of non-cardiac and cardiac comorbidities and mortality from HF at 6th month

Number of non-cardiac and cardiac comorbidities	Mortality from HF at 6th month				P
	No		Yes		
	N	%	n	%	
Non-cardiac					
0	38	12,2	2	20,0	0,803
1	78	25,0	2	20,0	0,991
> 1	196	62,8	6	60,0	0,879
Cardiac					
0	1	0,3	1	10,0	0,068
1	30	9,6	0	0,0	0,634
> 1	281	90,1	9	90,0	0,598

Table 24: Analysis of the relationship between number of non-cardiac and cardiac comorbidities and mortality from HF at 12th month

Number of non-cardiac and cardiac comorbidities	Mortality from HF at 12th month				P
	No		Yes		
	n	%	n	%	
Non-cardiac					
0	37	12,3	2	16,7	0,994
1	74	24,7	3	25,0	0,751
> 1	189	63,0	7	58,3	0,979
Cardiac					
0	1	0,3	0	0,0	**0,011**
1	**30**	**10,0**	**0**	**0,0**	**0,514**
> 1	269	89,7	12	100,0	0,498

Table 25: Analysis of the relationship between number of non-cardiac and cardiac comorbidities and mortality from HF at 24th month

Number of non-cardiac and cardiac comorbidities	Mortality from HF at 24th month				P
	No		Yes		
	n	%	n	%	
Non-cardiac					
0	34	11,8	4	23,5	0,298
1	72	25,1	2	11,8	0,342
> 1	181	63,1	11	64,7	0,900
Cardiac					
0	1	0,3	0	0,0	**0,034**
1	30	10,5	0	0,0	0,322
> 1	256	89,2	17	100,0	0,309

12. ANALYSIS OF RELATIONSHIP BETWEEN CLINICAL FEATURES AND REHOSPITALIZATIONS AND MORTALITY FROM HF.

There was significant relationship between rehospitalizations for HF at 3rd month and clinical features of the hospitalized patients for ECCHF with AH the time of hospitalization, type of the conduction abnormalities, LV EF and LF hypertrophy. When we evaluated rehospitalizations in the observed period we found significantly higher proportion of patients with LBBB (p=0,002), decreased EF < 40% (p=0,002) and without left ventricular hypertrophy (p=0,04) (fig. 20, fig. 21), (table 26)

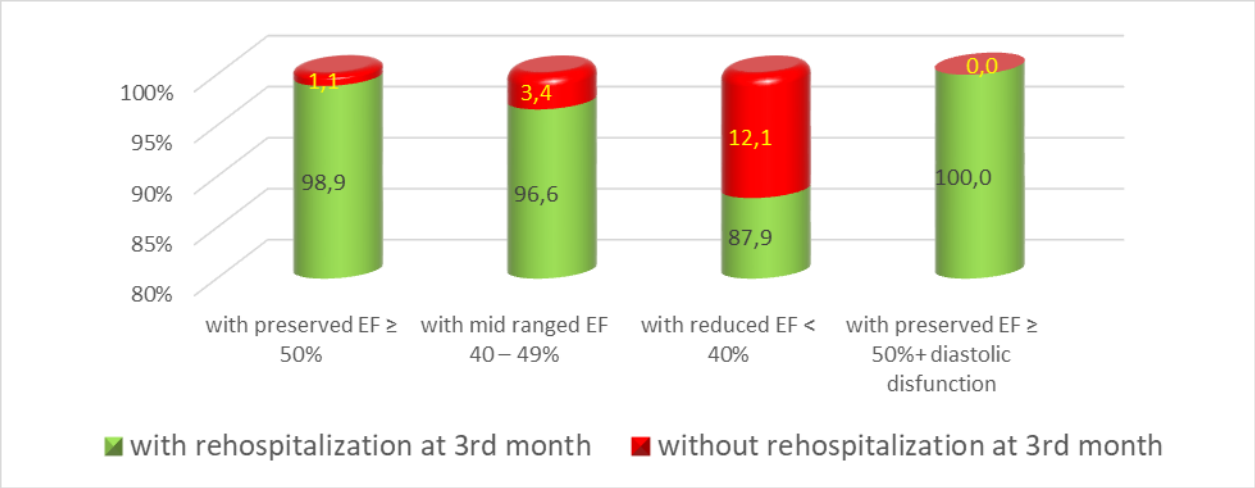


Figure 20: Distribution of patients by LV EF and rehospitalizations for HF at 3rd month

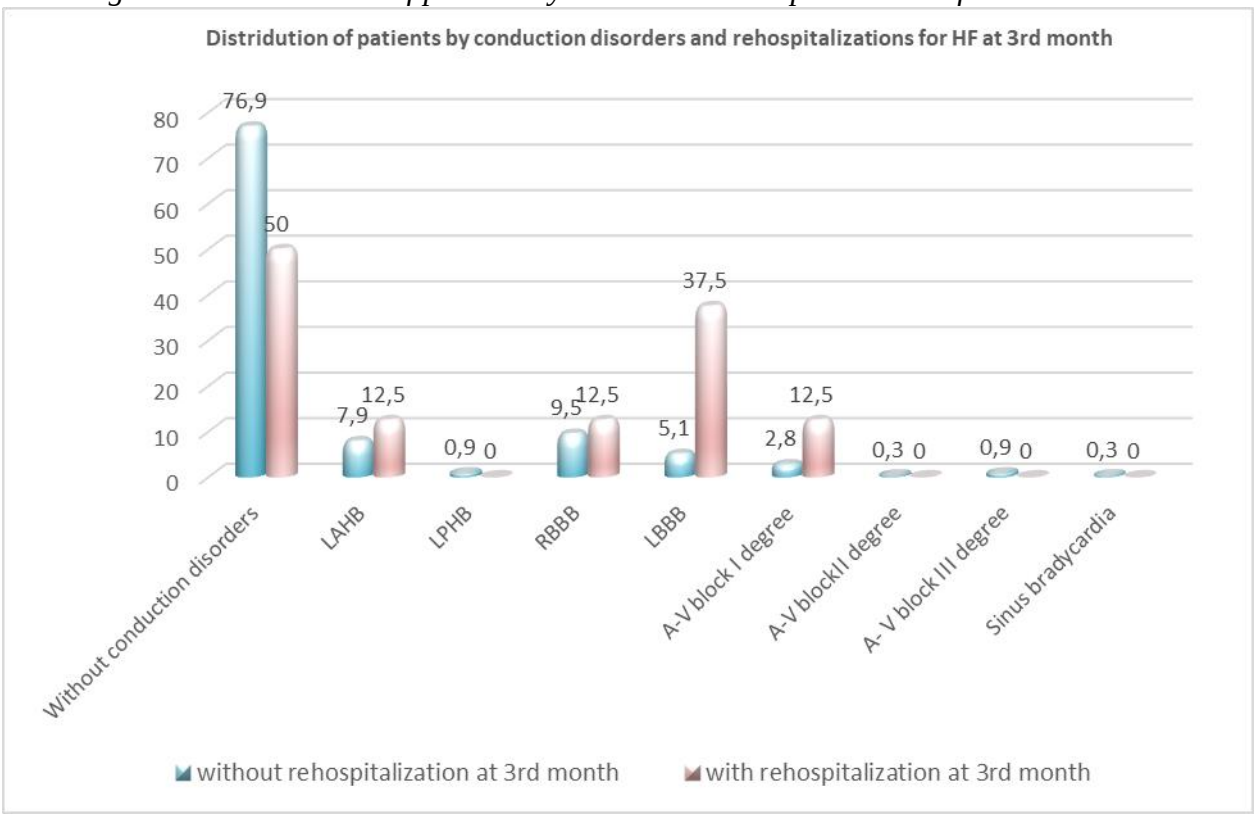


Figure 21: Distribution of patients by conduction abnormalities and rehospitalizations for HF at 3rd month

There was significant relationship between rehospitalizations at 6th month and clinical characteristics of the hospitalized patients for ECCHF only with the type of conduction abnormalities, non-rehospitalized patients for that period have significantly higher proportion of AV-block II degree and sinus bradycardia (fig. 22)

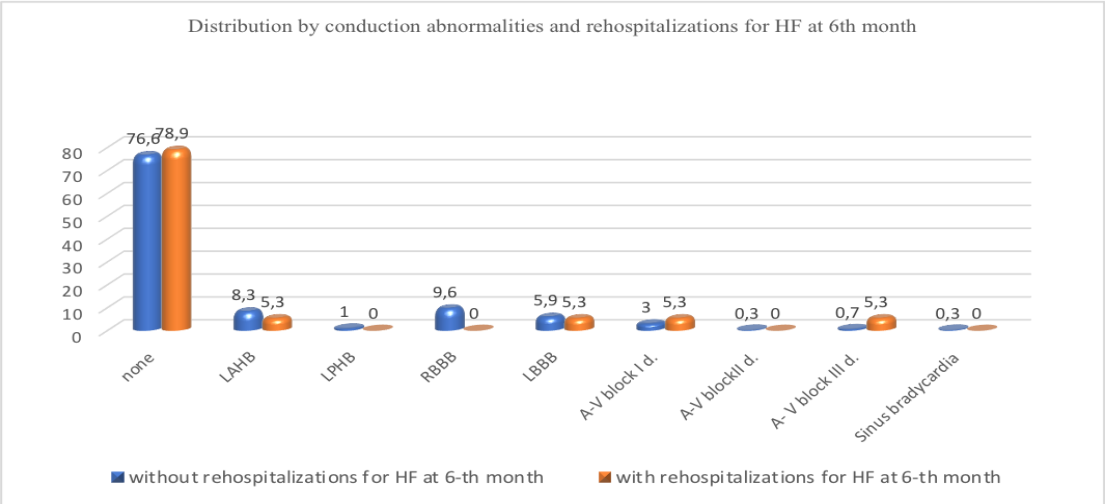


Figure 22: Distribution by conduction abnormalities and rehospitalizations for HF at 6th month.

There was no statistically significant relationship between rehospitalizations at 12th month and clinical characteristics of the hospitalized patients for ECCHF.

There was significant relationship between rehospitalizations for HF at 24th month and clinical features of the hospitalized patients for ECCHF with other complications, type of the conduction abnormalities and LV EF; the rehospitalized patients in this period of time have significantly higher proportion of pleural effusion (p=0,004) and reduced EF < 40% (p=0,002), while non-rehospitalized patients represent statistically significantly higher percentage of patients without complications and sinus bradycardia (fig. 23) (table 27).

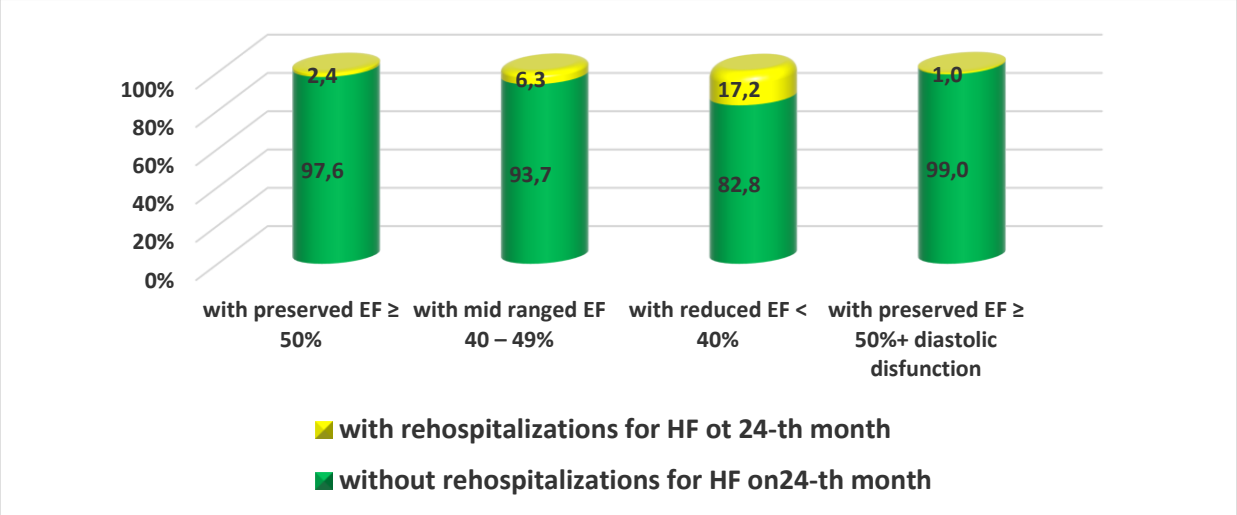


Figure 23: Distribution of patients by LV EF and rehospitalizations for HF at 24th month.

Table 26: Analysis of the relationship between clinical characteristics of the hospitalized patients for ECCHF and rehospitalizations for HF at 3rd month.

Clinical characteristics	Rehospitalizations for HF at 3rd month				P
	No		Yes		
	N	%	N	%	
Clinical criteria for ECCHF					
Fatigue during usual physical activities	124	39,2	1	12,5	0,244
Fatigue during mild physical activities	154	48,7	5	62,5	0,680
Dyspnea during usual physical activities	16	5,1	0	0,0	0,866
Dyspnea during mild physical activities	172	54,4	6	75,0	0,426
Dyspnea at rest	118	37,3	3	37,5	0,720
Arterial blood pressure during hospitalization (mmHg)					
Under 90	2	0,6	0	0,0	**0,033**
90-100	16	5,1	2	25,0	0,101
100-120	97	30,7	2	25,0	0,966
120-140	112	35,4	3	37,5	0,801
Above 140	89	28,2	1	12,5	0,562
NYHA Functional class (F.C.)					
II	52	16,5	1	12,5	0,856
III	254	80,4	7	87,5	0,961
IV	10	3,2	0	0,0	0,606
Type of arrhythmia					
None	233	73,7	5	62,5	0,762
Paroxysmal supraventricular tachycardia	4	1,3	0	0,0	0,202
Ventricular extrasystoles	38	12,0	0	0,0	0,627
Non-sustained VT	3	0,9	0	0,0	0,099
Supraventricular extrasystoles	17	5,4	0	0,0	0,900
Paroxysmal atrial fibrillation	37	11,7	3	37,5	0,100
Other complications					
None	293	92,7	7	87,5	0,898
Pleural effusion	17	5,4	0	0,0	0,900
Pericardial effusion	7	2,2	1	12,5	0,482
Ascites	4	1,3	0	0,0	0,202
Type of conduction abnormalities					
None	243	76,9	4	50,0	0,179
LAHB	25	7,9	1	12,5	0,852
LPHB	3	0,9	0	0,0	0,099
RBBB	30	9,5	1	12,5	0,746
LBBB	16	5,1	3	37,5	**0,002**
1st degree AV block	9	2,8	1	12,5	0,592
2nd degree AV block	1	0,3	0	0,0	0,002
3rd degree AV block	3	0,9	0	0,0	0,099
Sinus bradycardia	1	0,3	0	0,0	**0,002**
LV ejection fraction					
With preserved EF ≥ 50%	93	29,4	1	12,5	0,518
With mid-range EF 40 – 49%	85	26,9	3	37,5	0,792
With reduced EF < 40%	29	9,2	4	50,0	**0,002**
With preserved EF ≥ 50% + diastolic dysfunction	109	34,5	0	0,0	0,097
Left ventricular hypertrophy					**0,040**
No	85	26,9	5	62,5	
Yes	231	73,1	3	37,5	

Table 27: Analysis of the relationship between clinical characteristics of the hospitalized patients for ECCHF and rehospitalizations for HF at 24th month.

Clinical characteristics	Rehospitalizations for HF at 24th month				P
	No		Yes		
	N	%	n	%	
Clinical criteria for ECCHF					
Fatigue during usual physical activities	112	39,4	6	46,2	0,841
Fatigue during mild physical activities	138	48,6	6	46,2	0,909
Dyspnea during usual physical activities	14	4,9	0	0,0	0,884
Dyspnea during mild physical activities	159	56,0	7	53,8	0,897
Dyspnea at rest	105	37,0	6	46,2	0,706
Arterial blood pressure during hospitalization (mmHg)					
Under 90	2	0,7	0	0,0	0,151
90-100	15	5,3	1	7,7	0,800
100-120	84	29,6	4	30,8	0,828
120-140	105	37,0	3	23,1	0,469
Above 140	78	27,5	5	38,5	0,584
NYHA Functional class (F.C.)					
II	50	17,6	3	23,1	0,892
III	227	79,9	10	76,9	0,928
IV	7	2,5	0	0,0	0,725
Type of arrhythmia					
None	205	72,2	11	84,6	0,507
Paroxysmal supraventricular tachycardia	3	1,1	1	7,7	0,437
Ventricular extrasystoles	36	12,7	1	7,7	0,917
Non-sustained VT	3	1,1	0	0,0	0,312
Supraventricular extrasystoles	17	6,0	0	0,0	0,764
Paroxysmal atrial fibrillation	36	12,7	0	0,0	0,349
Other complications					
None	269	94,7	10	76,9	**0,042**
Pleural effusion	10	3,5	3	23,1	**0,007**
Pericardial effusion	5	1,8	0	0,0	0,547
Ascites	2	0,7	1	7,7	0,293
Type of conduction abnormalities					
None	222	78,2	8	61,5	0,285
LAHB	20	7,0	2	15,4	0,555
LPHB	1	0,4	1	7,7	0,172
RBBB	24	8,5	2	15,4	0,720
LBBB	17	6,0	1	7,7	0,732
1st degree AV block	8	2,8	1	7,7	0,856
2nd degree AV block	1	0,4	0	0,0	0,039
3rd degree AV block	3	1,1	0	0,0	0,312
Sinus bradycardia	1	0,4	0	0,0	**0,039**
LV ejection fraction					
With preserved EF ≥ 50%	83	29,2	2	15,4	0,446
With mid-range EF 40 – 49%	74	26,1	5	38,5	0,504
With reduced EF < 40%	24	8,5	5	38,5	**0,002**
With preserved EF ≥ 50% + diastolic dysfunction	103	36,3	1	7,7	0,069
Left ventricular hypertrophy					0,200
No	76	26,8	6	46,2	
Yes	208	73,2	7	53,8	

There was significant relationship between mortality from HF at 24th month and clinical characteristics of the hospitalized patients for ECCHF with LV EF and the type of conduction abnormalities.

The dead patients in this period of time had significantly higher proportion of LBBB (p=0,008) and AV block I degree (p=0,003) and reduced EF < 40% while survived patients are statistically significantly higher percentage preserved EF ≥ 50% and diastolic dysfunction (p=0,004) (table 28).

Table 28: Analysis of the relationship between clinical characteristics of the hospitalized patients for ECCHF and mortality from HF at 24th month.

Clinical characteristics	Mortality from HF at 24th month				P
	No		Yes		
	N	%	N	%	
Clinical criteria for ECCHF					
Fatigue during usual physical activities	113	39,4	8	47,1	0,708
Fatigue during mild physical activities	141	49,1	7	41,2	0,701
Dyspnea during usual physical activities	13	4,5	1	5,9	0,742
Dyspnea during mild physical activities	158	55,1	11	64,7	0,601
Dyspnea at rest	110	38,3	5	29,4	0,254
Arterial blood pressure during hospitalization (mmHg)					
Under 90	2	0,7	0	0,0	0,232
90-100	15	5,2	2	11,8	0,543
100-120	85	29,6	7	41,2	0,459
120-140	103	35,9	6	35,3	0,834
Above 140	82	28,6	2	11,8	0,220
NYHA Functional class (F.C.)					
II	52	18,1	1	5,9	0,237
III	228	79,4	16	94,1	0,244
IV	7	2,4	0	0,0	0,847
Type of arrhythmia					
None	211	73,5	12	70,6	0,984
Paroxysmal supraventricular tachycardia	3	1,0	1	5,9	0,524
Ventricular extrasystoles	36	12,5	1	5,9	0,669
Non-sustained VT	3	1,0	0	0	0,381
Supraventricular extrasystoles	17	5,9	0	0	0,627
Paroxysmal atrial fibrillation	*33*	*11,5*	*3*	*17,6*	*0,711*
Other complications					
None	270	94,1	14	82,4	0,165
Pleural effusion	12	4,2	2	11,8	0,392
Pericardial effusion	5	1,7	1	5,9	0,752
Ascites	2	0,7	1	5,9	0,751
Type of conduction abnormalities					
None	225	78,4	9	52,9	0,165
LAHB	22	7,7	1	5,9	0,841
LPHB	1	0,4	1	5,9	0,232
RBBB	27	9,4	2	11,8	0,917
LBBB	14	4,9	4	23,5	**0,008**
1st degree AV block	6	2,1	3	17,6	**0,003**
2nd degree AV block	1	0,4	0	0,0	0,054
3rd degree AV block	3	1,0	0	0,0	0,404
Sinus bradycardia	1	0,4	0	0,0	0,054
LV ejection fraction					
With preserved EF ≥ 50%	81	28,2	6	35,3	0,724
With mid-range EF 40 – 49%	76	26,5	5	29,4	0,984
With reduced EF < 40%	24	8,4	6	35,3	**0,001**
With preserved EF ≥ 50% + diastolic dysfunction	106	36,9	0	0,0	**0,004**
Left ventricular hypertrophy					0,091
No	76	26,5	8	47,1	
Yes	211	73,5	9	52,9	

13. ANALYSIS OF RELATIONSHIP BETWEEN THE EXAMINED BIOMARKERS AND REHOSPITALIZATIONS AND MORTALITY IN HF.

There is no significant association between values of biomarkers NT-proBNP, IL-18 and EF of LV and NYHA F.C. The available data do not give reason to claim statistical significance between the quantitative values of biomarkers and NT-proBNP, IL-18, Cystatin C and mortality from HF at 6th, 12th and 24th month.

We found a significant relationship between quantitative values of biomarkers NT-proBNP, IL-18, Cystatin C and HF rehospitalizations:

- **-The available data have statistical representativeness only for the rehospitalizations at 6th month;**
- **-The mean value of IL-18 at rehospitalized patients for this period is significantly higher than the non-rehospitalized (p=0,044)** (table 39) (fig. 24)

Table 29: Comparative analysis of relationship between quantitative values of biomarkers and NT-proBNP, IL-18, Cystatin C and rehospitalizations for HF at 6th month.

Biomarkers	**Rehospitalizations for HF at 6th month**						**P**
	No			Yes			
	n	$\overline{X}$	SD	n	$\overline{X}$	SD	
NT-pro BNP	67	10,10	8,53	9	10,90	9,68	0,917
IL-18	67	134,86	115,27	9	294,76	230,56	**0,044**
Cystatin C	67	1,48	0,63	9	1,68	0,70	0,484

The difference in mean values is statistically significant only in IL-18.

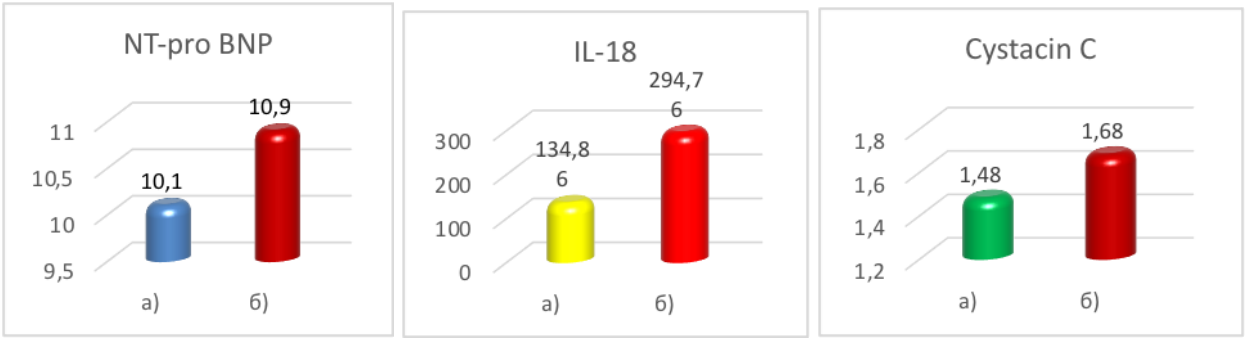
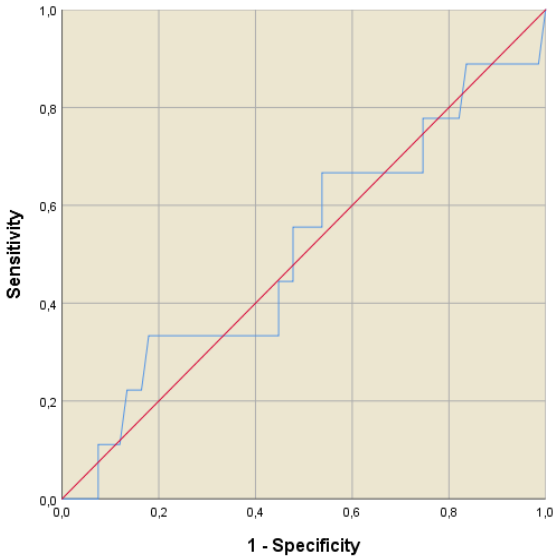


Figure 24 : Comparative analysis of relationship between quantitative values of biomarkers and NT-proBNP, IL-18, Cystatin C and rehospitalizations for HF at 6th month (A-No, B-Yes)

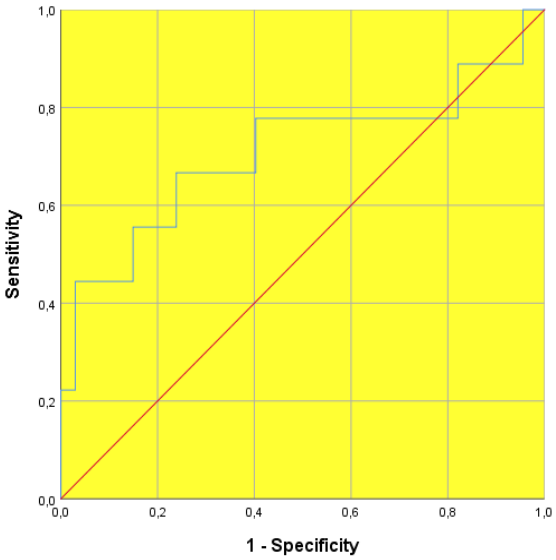
There is a representative group of nine cases for rehospitalizations because of HF at 6th month in our contingent. That is why it was possible to test the three biomarkers (in quantitative and categorical variant) as indicators individually and in combination for this event type only for this period

The statistical processing of the data from the studied contingent proves that only IL-18 has statistically significant cut-off value for delimitation of rehospitalized from non-rehospitalized patients at 6th month (fig. 25). Values of area under curve between 0.7 and 0.8 are considered like sufficient, which is also confirmed by the not so high values of validation criteria (table 30). The high percentage (94) of negative predictive value should be noted, which gives high probability of absence of rehospitalization in subjects with value under the cut-off point.

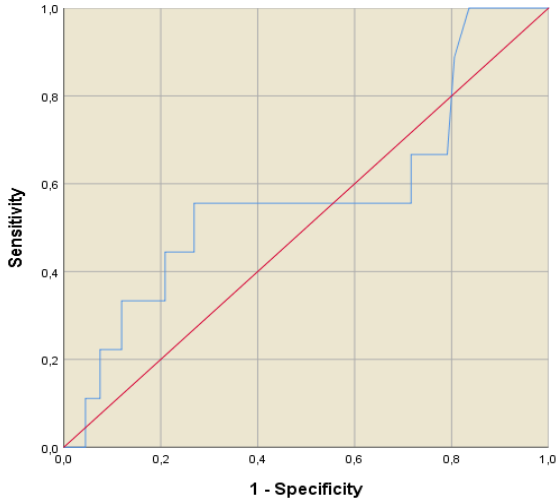
Figure 25: ROC curves for determination of cut-off point in delimitation of patients with rehospitalization from these without rehospitalization at 6th month of: a)NT-proBNP (area under curve 0.511, p=0,917); b)IL-18 (area under curve 0.708, p=0,044); c)Cystatin C (area under curve 0.572, p=0,484)



а) NT pro BNP area under curve 0,511, p=0,917)



б) IL-18 (area under curve 0,708), p=0,044),



в) Cystatin C (area under curve 0,572, p=0,484)

Figure 25: ROC curves for determination of cut-off point of biomarkers in delimitation of patients with rehospitalization from these without rehospitalization at 6th month

In order to assess quantitatively the association of the examined biomarkers with risk of rehospitaizations at 6th month we applied binary logistic regression, which showed the following results (table 30).

- **Only IL-18 has statistically significant odds ratio in comparison derived from the found cut-off point by ROC curve analysis;**
- **From this result it follows that the patients with value of IL-18 ≥ 219 have six times higher risk for rehospitalization until 6th month compared with patients with lower values (table 31).**

Table 30: IL-18 cut-off point for the delimitation of patients with rehospitalization from patients without rehospitalization at 6th month, and values of criteria for screening tests validation

Cut-off point	Sensitivity	Specificity	Positive predictive value	Negative predictive value	% Correct answers
≥219	**67**	**76**	**27**	**94**	**75**

Таблица 31: Odds ratio and 95% CI of the examined biomarkers, as indicator for rehospitalization at 6th month

Indicator	Comparison	OR	95% CI		P
			Lower border	Upper border	
NT pro BNP	Increased / Normal	0,79	0,179	3,492	0,758
IL 18	Increased / Normal	4,32	0,834	22,333	0,081
Cystatin C	Increased / Normal	246428432,34	0,00	.	0,999
IL 18	≥ 219 / < 219	6,37	1,43	28,44	**0,015**

IL-18 takes part in both: the pathogenesis of CHF and its specific functions are an object of studies, and there is evidence that the proinflammatory cytokines play role in the pathogenesis of the advanced HF (Nakanishi K.), (Okamura H.). The results from our study confirmed these data from literature and we also found that the increased serum levels of IL-18 over determined value in hospitalized patients with ECCHF are indicative for significantly higher risk for rehospitalizations because of exacerbated CCHF during the first six months. We also found a cut-off point of IL-18 over which the risk for rehospitalizations is increased more than six times.

14. COMPARATIVE ANALYSIS OF COMORBIDITY, REHOSPITALIZATIONS AND MORTALITY IN MEN AND WOMEN

There is statistically significant difference by gender regarding the number of non-cardiac comorbidities in the examined contingent. The percentage of patients with one non-cardiac comorbidity is significantly higher in women, whereas the percentage of patients with four and five non-cardiac comorbidities is significantly higher in men. There is no significant difference between men and women regarding cardiac comorbidity. We did not find a significant difference between men and women in HF mortality and hospitalizations due to HF deterioration.

15. COMPARATIVE ANALYSIS OF NON-CARDIAC COMORBIDITY IN PATIENTS WITH HF WITH PRESERVED EF BY GENDER

Data of patients with HFpEF were additionally analyzed, because they represent the largest number (205) of the examined contingent.

Regarding non-cardiac comorbidity in our contingent with HFpEF we found statistically significant difference between men and women of the frequency of the thyroid pathology, anemia and CKF. There was higher frequency of anemia ($p<0,001$) and thyroid pathology ($p=0,01$) in women, while CKF predominates in men ($p=0,003$) (fig. 26). There was no significant difference in the frequency of the rest examined non-cardiac comorbidities, as we found a tendency for higher frequency of hyperuricemia and gout in men.

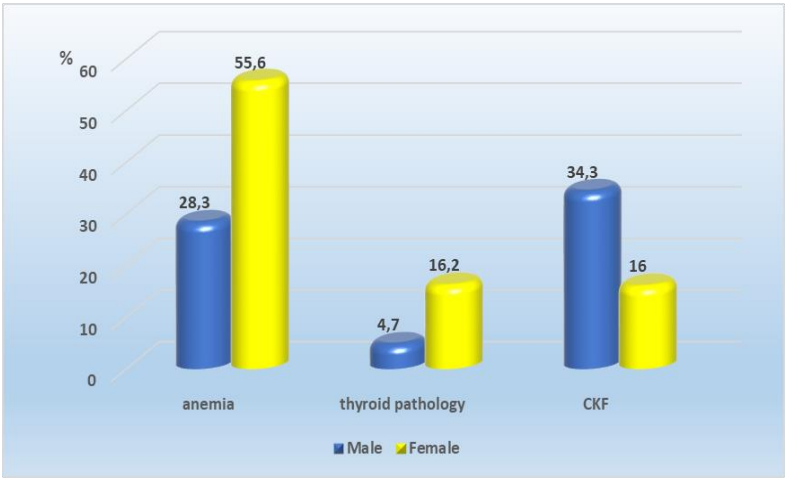


Figure 26: Comparative analysis of men and women regarding non-cardiac comorbidity in patients with HFpEF – comorbidities by which men and women differ significantly ***(p<0,05)***

Regarding cardiac comorbidity the frequency did not differ significantly in men and women in patients with HFpEF and patients with IHD as well and frequency of ACS and MI is comparatively higher in men (fig. 27).

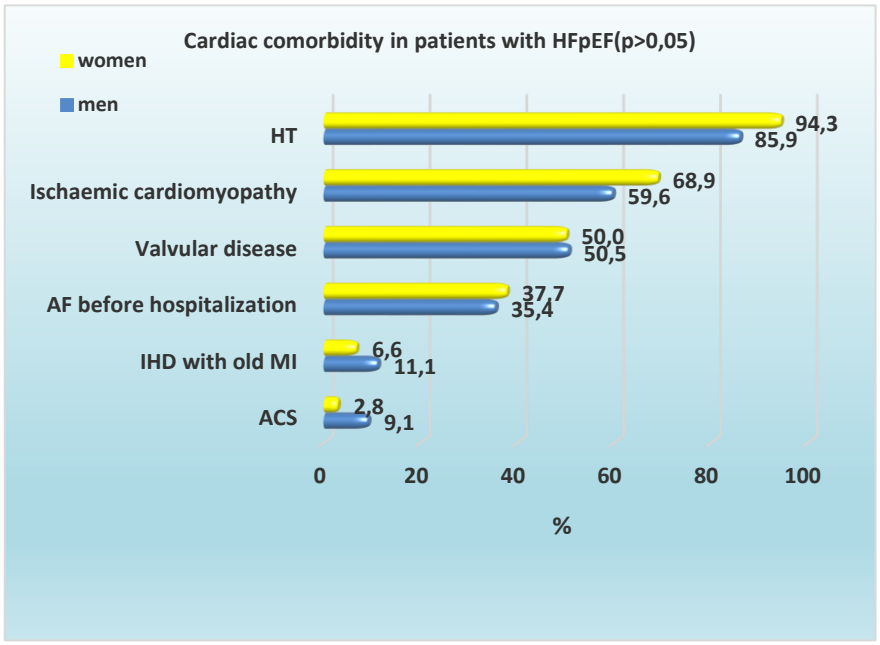


Figure 27: Comparative analysis of men and women regarding cardiac comorbidity in patients with HFpEF

SURVIVAL

We applied additional statistical tests in order to analyze the survival rate:

1. ***Kaplan-Meier method*** *– for estimation of the time until onset of the examined event Kaplan-Meier product limit estimation of the survival function.*
2. ***Log-rank, Breslow and Tarone-Ware tests*** *- for estimation of the influence of the examined factors on onset of the examined event*
3. ***Cox-regression analysis*** *for quantitative estimation of the influence of the examined factors on onset of the examined event*
4. ***ROC curve analysis*** *for cut-off point in delimitation of patients with rehospitalization from these without rehospitalization.*

Overall survival of the examined contingent

The mean total survival rate is 22,26 ± 0,28 months in 95% OR from 21,70 to 22,82 months.

Table 32 represents the total survival calculated by the method of Kaplan-Meier. The most important moments in it are:

- ***45 (13.7%) from 328 patients followed-up for total survival died from HF and other reasons;***
- ***The highest mortality rate is observed during the first six months – 15 cases or 1/3 from the total number of dead in the period of follow-up 45 patients;***
- ***The second mortality rank was taken by the second six months, when 14 (31.1%) of the exitus cases occurred;***
- ***The minimally registered survival was one week and the maximal – 2 years;***
- ***The six months survival is 95.4%, one year – 91.2%, 18-months -89.3% and two years survival – 86.3%.***

Table 32: Total survival rate of the examined contingent

Period (months)	Cumulative survival	Standard error	Number of exitus cases	Cumulative number of exitus cases	Number of remaining patients
0	1,000		0	0	328
0,25	0,994	0,004	2	2	326
3	0,982	0,007	4	6	322
6	0,954	0,012	9	15	313
9	0,936	0,014	6	21	307
12	0,912	0,016	8	29	299
15	0,905	0,016	2	31	297
18	0,893	0,017	4	35	293
21	0,881	0,018	4	39	289
24	0,863	0,019	6	45	0

Figure 28 shows the curve of the cumulative probability of survival. The so- called discontinuous cases involving patients who survived in the end of the follow-up period were marked with a vertical line. The steeper section of the graph is in the first year of follow-up. Than the declining cumulative survival becomes slightly smoother and it is 0,85 at 24th month.

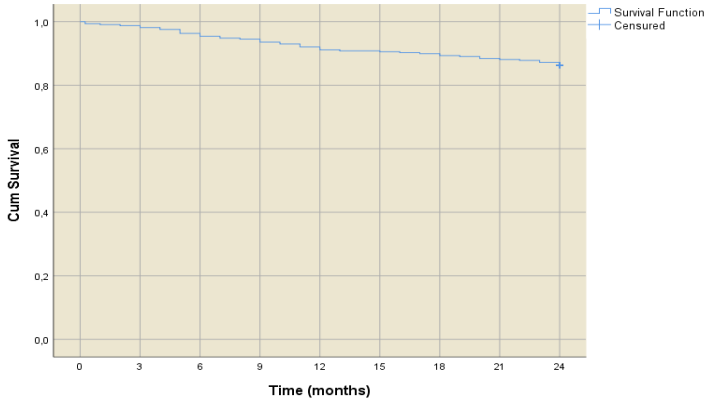


Figure 28: Overall survival by Kaplan-Meier

Factors influencing the total survival

The next study stage included analysis of the probable factors influencing the examined type of survival. For this purpose the Kaplan-Meier method was applied again and the assessment of impact was performed with Log-rank, Breslow and Tarone-Ware tests.

The following indicators were tested: gender, age, overweight, cause, comorbidity, and EF.

Gender

Kaplan-Meier survival analysis shows about half a month longer mean survival rate of women than men, but Log-rank, Breslow and Tarone-Ware found that the difference was not significant (table 33).

Table 33: Comparative analysis of total survival rate between men and women

Gender	Number of cases	Number of events	Mean survival (months)*	Standard error	95% CI	
					Lower border	Upper border
Men	186	28	22,04	0,39	21,28	22,80
Women	142	17	22,56	0,43	21,71	23,40

* Log Rank (Mantel-Cox) p = 0,422

Fig. 29 shows that cumulative survival in men after 9th month decreases faster than women and it reaches lower values, but there is no significant difference between them.

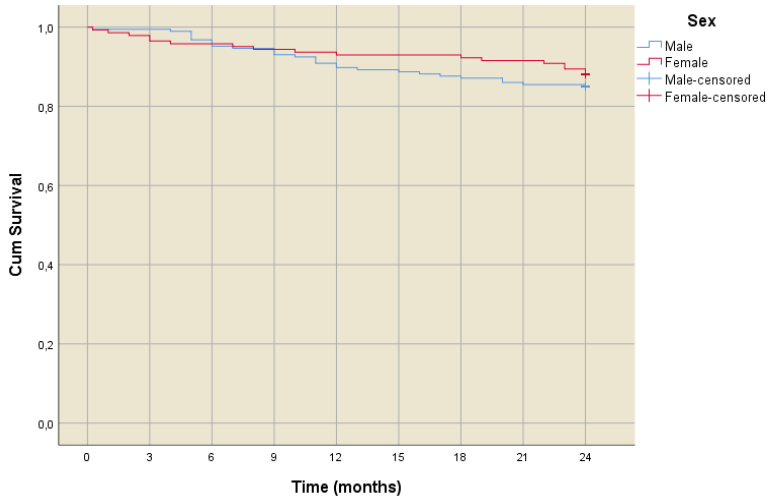


Figure 29: Total survival of male and female patients

Age

Since the age is quantitative feature ROC curve was applied in order to estimate whether statistically significant cut-off point exists. The results (fig. 30) show that statistically significant cut-off point can not be detected.

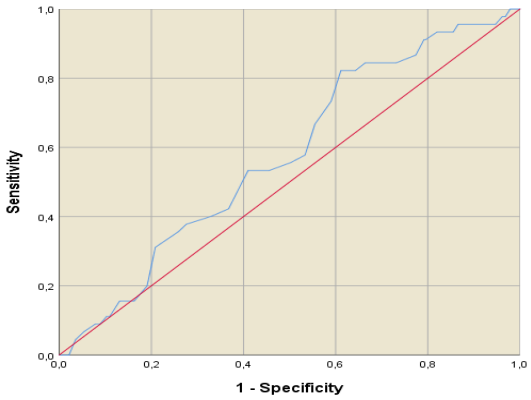


Figure 30: ROC curve of age for detection of cut-off point in delimitation of dead from survived patients (area under curve 0.577, p=0,098).

Overweight

The performed analysis of survival according to the Kaplan-Meier method and Log-rank, Breslow and Tarone-Ware tests found statistically negligible difference of survival between overweight and non-overweight patients (table 34). This is confirmed by figure 31.

Table 34: Comparative analysis of total survival according to the indicator overweight

Overweight	Number of cases	Number of events	Mean survival (months)*	Standard error	95% CI	
					Lower border	Upper border
No	181	23	22,13	0,41	21,33	22,94
Yes	147	22	22,42	0,39	21,65	23,20

* Log Rank (Mantel-Cox) p = 0,617

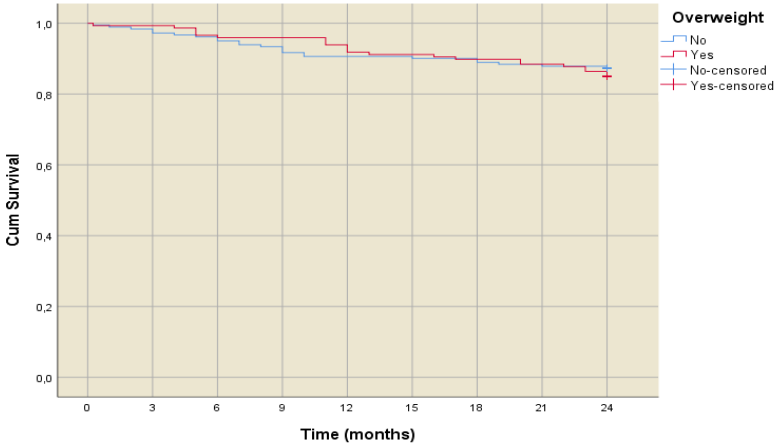


Figure 31: Total survival of patients according to the indicator overweight

Causes of death

The performed analysis of survival according to the Kaplan-Meier method and Log-rank, Breslow and Tarone-Ware tests found that the difference between the mean survival of the patients died from the two types of causes is not statistically significant (table 35).

Table. 35: Comparative analysis of total survival according to the indicator causes of death

Causes of death	Number of cases	Number of events	Mean survival (months)*	Standard error	95% CI	
					Lower border	Upper border
HF or other CVD	40	40	11,34	1,18	9,03	13,64
Other	5	5	11,40	2,91	5,70	17,10

* Log Rank (Mantel-Cox) p = 0,749

Non-cardiac comorbidity

The performed analysis of survival according to the Kaplan-Meier method and Log-rank, Breslow and Tarone-Ware tests found that the difference between the mean survival of the patients with different non-cardiac comorbidity is not statistically significant (table 36). This is shown in figure 32.

Table 36: Comparative analysis of total survival according to the indicator non-cardiac comorbidity

Non-cardiac comorbidity	Number of cases	Number of events	Mean survival (months)*	Standard error	95% CI	
					Lower border	Upper border
None	41	7	21,95	0,83	20,33	23,57
One	81	9	22,40	0,61	21,19	23,60
Two or more	206	29	22,27	0,36	21,58	22,97

* Log Rank (Mantel-Cox) p = 0,664

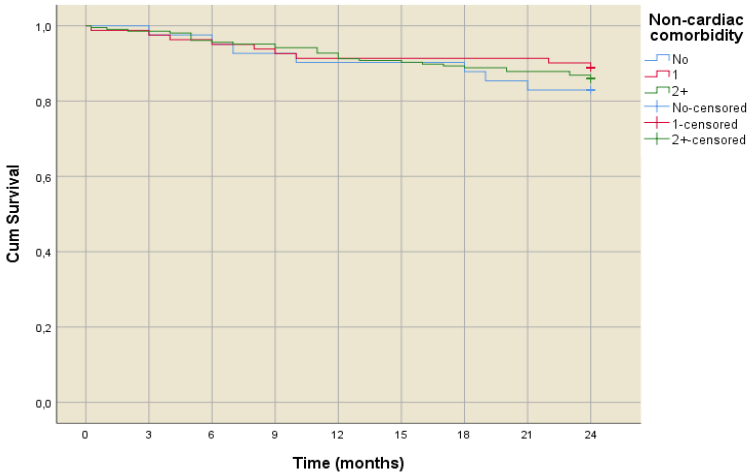


Figure 32: Total survival of patients according to the indicator non-cardiac comorbidity

Cardiac comorbidity

Almost all patients have at least one STATISTICALLY SIGNIFICANT cardiac disease. That is why the can not be divided in two categories – with and without CVD (table 37).

Table 37: Comparative analysis of general survival according to the indicator cardiac comorbidity

Cardiac comorbidity	Number of cases	Number of events	Mean survival (months)*	Standard error	95% CI	
					Lower border	Upper border
None	2	1	14,00[a]	7,07	0,14	27,86
One	31	1	23,26[b]	0,73	21,83	24,69
Two or more	295	43	22,22[b]	0,30	21,63	22,80

* Log Rank (Mantel-Cox) p = 0,033

** the same letters mean no significant difference, while the different - presence of such (p<0,05)

Ejection fraction

The performed analysis of survival according to the Kaplan-Meier method and Log-rank, Breslow and Tarone-Ware tests found that (table 38):

- Patients with reduced EF < 40% have statistically significant lowest mean survival, whereas patients with preserved EF ≥ 50% + diastolic dysfunction have statistically significant highest mean survival.
- The other two categories – with preserved EF ≥ 50% and mid-range EF 40–49% had intermediate values of the mean survival (statistically insignificant).
- Fig. 33 shows these results.

Table 38: Comparative analysis of total survival according to the indicator EF

EF	Number of cases	Number of events	Mean survival (months)*	Standard error	95% CI	
					Lower border	Upper border
With preserved EF ≥ 50%	95	15	21,86[a]	0,61	20,66	23,06
With mid-range EF 40 – 49%	88	13	22,38[a]	0,53	21,34	23,41
With reduced EF < 40%	35	12	20,18[b]	1,11	18,00	22,36
With preserved EF ≥ 50% + diastolic dysfunction	110	5	23,18[c]	0,37	22,47	23,90

*** Log Rank (Mantel-Cox) p < 0,001**

**** the same letters mean no significant difference, while the different - presence of such (p<0,05)**

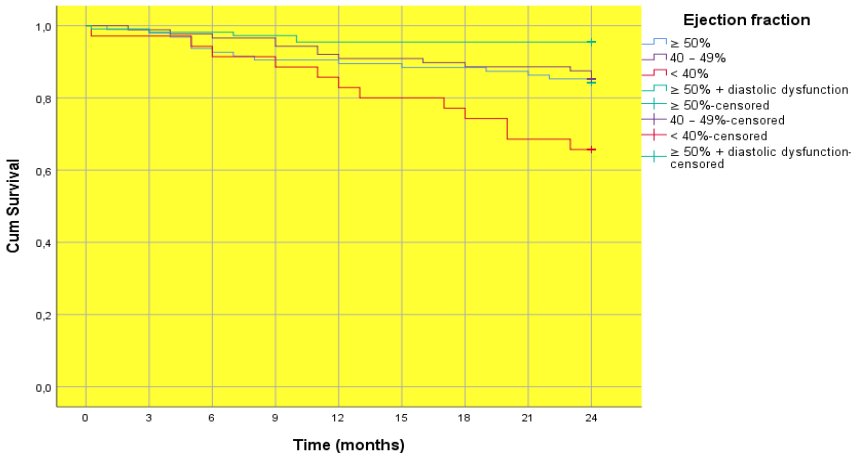


Figure 33: General survival of patients according to EF

Quantitative assessment of the factors associated with the occurrence of exitus

The performed analyses estimated that ejection fraction is significantly associated factor with occurrence of exitus. In order to assess the quantitative impact of the different categories of COX-regressive analysis was performed. Preserved EF ≥ 50% + diastolic dysfunction was used as basis for comparison. The largest proportion of patients with HFpEF ≥ 50% + diastolic dysfunction are patients with arterial hypertension as cardiac comorbidity without any other cardiac and non-cardiac comorbidities. A large proportion of patients with HFpEF ≥ 50% included in the study are polymorbid with two and three non-cardiac comorbidities.

Table 39 shows that:

- Three from the categories have significantly different risk for exitus in comparison with the base;
- Patients with preserved EF ≥ 50% have 3,7 higher times increased risk for exitus than the base category;
- Patients with mid-range EF 40-49% have approximately 3,4 higher times increased risk for exitus compared with the base category;
- Patients with reduced EF < 40% have approximately 8,6 higher times increased risk for exitus than the base category.

Table 39: Odds ratio and 95% CI of ejection fraction as a factor for exitus

Indicator	Comparison	HR	95% CI		P
			Lower border	Upper border	
Ejection fraction	Preserved EF ≥ 50% / Preserved EF ≥ 50% + diastolic dysfunction	3,68	1,34	10,11	0,012
	Mid-range EF 40 – 49% / Preserved EF ≥ 50% + diastolic dysfunction	3,35	1,20	9,41	0,021
	Reduced EF < 40% / Preserved EF ≥ 50% + diastolic dysfunction	8,55	3,01	24,28	<0,001

When we analyzed the results from our prospective study of comorbidity, clinical course, instrumental examination, biomarkers in the hospitalized patient with HF, rehospitalizations and mortality follow-up for 24 months period, the risk profile of patients was modeled.

According to the risk of rehospitalizations and death patients with HF were differentiated into 3 groups: high risk group, intermediate risk group and low risk group for rehospitalizations and death.

The following factors are associated with higher risk for rehospitalizations and death are: ischemic heart disease; previous myocardial infarction; type II diabetes mellitus with duration more than 5 years; gout; atrial fibrillation; left bundle branch block; AV block I degree; increased IL-18 ≥ 219 pg/ml; HFrEF – EF < 40% and complicated course of HF during the hospitalization (pleural effusion, pericardial effusion, ascites) -**High risk group**.

The Intermediate risk group includes patients with: HFmrEF; EF 40—49%; sinus bradycardia; COPD; anemia and patients with smaller number of cardiac comorbidities and with uncomplicated clinical course of HF during the time of hospitalization.

The Low risk group of patients have better prognosis and lower frequency of rehospitalizations for HF and this group of patients is with: smaller number of non-cardiac and cardiac comorbidities; sinus rhythm; without rhythm and conduction abnormalities; IL-18 < 219 pg/ml; HFpEF – EF ≥ 50%; diastolic left ventricular dysfunction.

Patients with higher risk for rehospitalizations are subject to complex HF treatment, comorbidities treatment and close monitoring after dehospitalization in order to ameliorate the prognosis.

VI. DISCUSSION

The current study included 328 patients hospitalized with ECCHF for the period of 2016-2018, the concomitant cardiac and non-cardiac diseases were analyzed, symptoms, clinical manifestation of HF, assessment of NYHA F.C., LV EF with echocardiography and patients' renal function. Three biomarkers were examined in 78 patients: NT-proBNP – marker for the myocardial stress, "gold standard" in HF diagnosis, Cystatin C and eGFR MDRD for accurate assessment of early renal injury (RI) in the course of HF interleukin-18 (IL-18), which has cardio depressive effect and it is also marker for inflammatory activity of the organism and apoptosis of cardiomyocyte. The examined contingent included 328 patients, 186 (56.7%) men and 142 (43.3%) women. The mean age of study participants is 66,55 ± 10,31 years with range 30-89 years. The age group 60-69 showed highest number (58) in men, followed by 56 patients in the age group 70-79. The age group 70-79 years showed highest number (50) in women, followed by 60-69. In our contingent the older patients over 60 years predominate, the highest frequency of HF is registered in men in 60-69 years, with one decade earlier in comparison with women. According to Gottdiener J S et al., who investigated the population risk for HF development, the frequency of CHF is high in elderly subjects and it majorly associated with age, gender and comorbidity.

Patients were prospectively followed-up regarding rehospitalizations and mortality for 24 months period after dehospitalization. The symptoms and manifestation of ECCHF was monitored and we found that at hospitalization the highest relative proportion of clinical criteria for ECCHF is nocturnal dyspnea (56.4%) followed by dyspnea in mild physical activities (54.3%). In 29 patients were observed pleural effusions, pericardial effusion and ascites as a manifestation of decompensation of heart failure, with the highest percentage (5.2%) is pleural effusion, followed by pericardial effusion with 2.4%. During the hospitalization patients with systolic blood pressure (SBP) 120-140 mm/Hg (35.1%) predominate. The distribution of patients according to the NYHA functional class of HF at hospitalization in the clinic is: patients with NYHA III F.C. showed highest relative proportion (80.2%), followed by 16.8% with NYHA II F.C. and the least number of cases were with NYHA IV F.C. (3%).

Patients with arrhythmias are 26.5% and the most common arrhythmia is paroxysmal atrial fibrillation (12.5%) followed by ventricular extrasystoles – 11.6% and non-sustained

ventricular tachycardia (VT) – 0.9%. Patients with conduction abnormalities are 23.8% with the highest number of subjects with right bundle branch block (RBBB) – 9.5%, left anterior hemiblock (LAHB) – 7.9%, followed by left bundle branch block (LBBB) – 6.1%. Approximately 72% from the study participants have left ventricular hypertrophy as we did not find significant relationship between LV hypertrophy and duration of AH (p=0,44).

We determined systolic and diastolic LV function with echocardiography, Doppler and TDI. Since all patients with diastolic dysfunction have also preserved EF ≥ 50% but not all patients with preserved EF ≥ 50% have diastolic dysfunction, we differentiated four patients' categories: subjects with preserved EF ≥ 50%; subjects with preserved EF ≥ 50% and diastolic dysfunction; subjects with mid-range EF 40-49% and subjects with reduced EF < 40%. Patients with preserved EF ≥ 50% showed highest relative proportion (62.5%), followed by patients with diastolic dysfunction – 33.5% and the smallest number of cases were with EF < 40% (10.7%). When we analyze the distribution of patients by NYHA F.C. in the different categories of LV EF we found significant difference only in patients with reduced EF < 40% - the relative proportion of II F.C. is significantly lower (p=0,05) than class III and IV which did not show significance with each other. When the four examined EF categories were analyzed, NYHA III F.C. showed highest relative proportion, followed by F.C. II in most cases (with the exception of cases with reduced EF) and on last place was F.C. IV. According to *Angerman CE* despite congestive heart failure is a syndrome which traditionally assumed as hemodynamic disorder, a weak correlation is often observed between the determined heart ejection fraction and CHF symptoms in some patients, which is confirmed by our study. The mechanism of reduced tolerance in physical activity in patients with CHF is not fully understood. For example severely impaired left ventricular function is sometimes detected in completely asymptomatic patients, while patients with preserved systolic function can express disabling CHF symptoms (*ADHERE*).

There is evidence that cardiovascular comorbidity in HF is: ischemic heart disease (IHD), arterial hypertension (AH), valve abnormalities, atrial fibrillation (AF) (Angermann CE), (Cheng RK).

The most common cardiac comorbidities in the examined patients with HF by us are: IHD, AH, valve heart abnormalities and AF. A number of authors focus on the polymorbidity of patients with HF (*Chamberlain AM, Chang RK, Levy D, Lloyd-Jones DM*). In the contingent of hospitalized patients with HF examined by us regarding cardiac pathology patients with more than two cardiac comorbidities were more commonly observed, with the highest relative proportion (56.7%) of patients with three cardiac comorbidities followed by two (22.6%). According to Gottdiener et al., the population risk for HF development is relatively high in the presence of coronary artery disease (CAD) (13.1%), SBP ≥ 140 mm/Hg (12.8%), increased level of CRP (9.7%) as authors indicate that the CHF frequency is high in elderly subjects and it is majorly associated with age, gender, clinical and subclinical CAD, increased SBP and with inflammatory process.

According to *Page RL 2nd* and *Lindenfelt J.* over 40% of patients with HF have five or more chronic comorbidities. Data of patients from the System Medicare USA show that in 40% of patients with HF were registered more than five non-cardiac comorbidities. The hospitalizations of these patients for ECCHF are much more common and their treatment in hospital is longer. The included patients with HF in our study are polymorbid, over 50% of patients are with 3 and more cardiac comorbidities. The percentage of non-cardiac comorbidity of the examined patients is also high, approximately ¼ of them are with 2 and 3 non-cardiac comorbidities. Patients with 2 non-cardiac comorbidities were the highest relative proportion (25.3%), followed by these with one non-cardiac comorbidity (24.7%). Our results confirm data of Metra M. et al., that the most common non-cardiac comorbidities and conditions in patients with HF are: type II diabetes mellitus (TD2M), chronic obstructive pulmonary disease (COPD), anemia, gout, thyroid pathology, obesity and chronic kidney failure (CKF). The non-cardiac comorbidity frequency in our study is: diabetes mellitus –

35.67%; anemia – 41.16%; overweight – 44.21%; gout – 18.29%; thyroid pathology – 14.02%; COPD – 25.3%; CKF – 26.5%.

We found the unfavorable effect of polymorbidity on HF long-term prognosis. The relative proportion of the survived patients until 12th and 24th month is significantly higher in patients without cardiac comorbidity. Patients without cardiac comorbidity are with significantly higher relative proportion of survived until 12th (p=0,011) and until 24th month (p=0,034). When we followed-up our patients we found that the mortality in patients with HF and chronic IHD and previous MI is significantly higher at 24th month (p=0,019). There was a statistically significant relationship between the higher HF mortality until the end of the second year and the permanent AF in the hospitalized patients (p=0,003). AF is the most common arrhythmia in HF independently of the concomitant LV EF as it increases the risk of thromboembolic complications (especially stroke) and it can disturb the cardiac function resulting in deterioration of HF symptoms. The incident HF induced by AF is associated with better prognosis, but the first detected episode of AF in patients with diagnosed HF and is associated with worse prognosis, probably because it is a marker for advanced disease and it also alters the cardiac function. According to *Sharma AK* et al., AF and CHF are very common conditions, which predispose each other, have common risk factors and they are also reason for morbidity and mortality in the general population. These conditions have common pathophysiology which includes structural and electrical remodeling dysregulation of the intracellular calcium and neuroendocrine mechanisms and common genetics. Despite the better survival in patients with restored sinus rhythm in comparison with the patients who remain in AF with rate control, the strategy for rhythm control does not have many advantages than the strategy for rate control. According to the *Swedberg K.* et al., patients with chronic CF and permanent AF have worse prognosis than these in sinus rhythm and this is explained by the advanced age and the severity of HF.

We found a significant relationship between HF mortality at 24th month and clinical characteristics of the patients hospitalized for ECCHF and the type of conduction abnormalities and LV ejection fraction. The patients who died in this period of time have significantly higher relative proportion of LBBB (p=0,008), AV block I degree (p=0,003) and reduced EF < 40% (p=0,001), while the survived are with statistically significantly higher percentage of preserved EF ≥ 50% and diastolic dysfunction (p=0,004). *Zannand F.* summarizes literature data which show that LBBB via intraventricular asynchrony mediation results in deterioration of heart function, cardiac remodeling and HF.

Regarding the influence of non-cardiac comorbidity on the mortality in the examined contingent we observed significantly higher mortality at 12th month in patients with TD2M with duration more than 5 years (p=0,011) and in patients with concomitant hyperuricemia and gout (p=0,013). Patients with HFpEF have higher comorbidity than patients with HFrEF and probably this is one of the reasons for this syndrome progression. According to *Gilbert RE* et al., the impaired carbohydrate tolerance and DM are seen more often in HF, as DM determines a worse functional status of patients and worse prognosis. The blood DM control is important factor for reduction of morbidity and mortality in patients with HFrEF. In these patients, DM majorly increases the risk of cardiovascular death and rehospitalization for HF in comparison with patients with HFrEF. The analysis of *OPTIMIZE-HF* report that patients with DM do not have increased risk of mortality and rehospitalizations in contrast with the higher risk of patients with DM with HFrEF. It was recently highlighted that hyperuricemia is a sign of impaired metabolic balance and hyperuricemia is independent marker for worse prognosis in CHF *(Pascual-Figal DA)*, which is confirmed by our study. When we analyzed data of the patients from the examined contingent by us we found that the one year mortality is significantly higher (p=0,013) in patients with concomitant hyperuricemia and gout. Data from *Framingham study* showed the relationship between hyperuricemia and the risk for

cardiovascular diseases and mortality, but the role of uric acid independently from the other established factor is not clear.

Regarding the non-cardiac comorbidity in patients with HFpEF from our contingent we found statistically significant difference of frequency of the thyroid pathology, anemia and CKF between men and women. Anemia ***(p<0,001)*** and thyroid pathology ***(p=0,01)*** are with higher frequency in women, as patients with CKF ***(p=0,003)*** predominate in men. There was not statistically significant difference in the distribution of the other studied non-cardiac comorbidities. According to *OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Patients with Heart Failure)* anemia frequency is higher in women with HF. Moreover patients with lower hemoglobin levels are elderly and more often with preserved left ventricular function. According to *CHARM (Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity)* the lower hemoglobin levels are associated with higher EF. Furthermore female gender, DM and worsening renal function are strong predictors for anemia manifestation.

The highest number of rehospitalizations (36) in our study was at 6th month from the follow-up and least number (12) – at 3rd month and during the four periods of follow-up as deterioration of HF is predominant reason for the rehospitalization. The deterioration of HF showed highest percentage (81.3%) in rehospitalizations at 24th month and lowest percentage – at 6th month – 52.8%.

The increase in the number of cardiac comorbidities is associated with higher percentage rehospitalizations because of HF deterioration, while in non-cardiac comorbidity this tendency is not clearly expressed. The only significant difference in the relative proportions of non- and rehospitalized patients is found in the rehospitalizations for HF at 24th month (p=0,039) in patients without cardiac comorbidity, as the percentage of non-rehospitalized from them is significantly higher. Regarding the rehospitalizations for HF we did not find significant difference between the observed cardiac and non-cardiac comorbidities in the study and the frequency of rehospitalizations for HF until 24th month as in patients with COPD, and also in these subjects with anemia we registered tendency for higher frequency of rehospitalizations for ECCHF. There is also higher frequency of rehospitalizations for ECCHF in patients with AF, with chronic IHD, as well presence of LBBB. **There was a significant relationship between rehospitalizations for HF at 3rd month and clinical characteristics of the hospitalized patients for ECCHF with blood pressure, type of conduction abnormalities, LV ejection fraction and left ventricular hypertrophy. The rehospitalized patients in this period of time have significantly higher relative proportion of LBBB (p=0,002), reduced EF < 40% (p=0,002) and absence of left ventricular hypertrophy (p=0,04)**. According to *Sharma* et al., the number of non-cardiac comorbidities increases with time, which is associated with worsening of the prognosis of patients with HF. That is why there is a need for development of strategy for treatment of non-cardiac comorbidity in patients with HF with aim to improve prognosis. Regarding the cardiac comorbidities authors reported tendency for increasing the frequency of atrial fibrillation (AF) from 32% to 37%, AH from 73% to 84% and dyslipidemia from 35% to 54%. The frequency of coronary artery disease (CAD) is likely reduced from 51% to 49% and periphery artery disease is almost unchanged (12%).

We found significant relationship between rehospitalizations for HF at 24th month and clinical characteristics of the hospitalized patients for ECCHF with the other complications (p=0,042), the type of the conduction abnormalities and LV ejection fraction.

Patients who were rehospitalized in this period of time have significantly higher relative proportion of pleural effusion (p=0,042) and reduced EF < 40% (p=0,002), while

the non-rehospitalized subjects are with statistically significantly higher percentage without complications (p=0,007) and sinus bradycardia.

Patients with HFpEF are more often hospitalized because of other reason, not for HF, while the frequency of hospitalizations for HF is higher in patients with HFrEF, which is in agreement with scientifical literature data *(Berry C.).*

We examined NT-proBNP in 78 from the hospitalized patients for conformation of HF diagnosis. The natriuretic peptides BNP, NT-proBNP indicate myocardial remodeling and myocardial stress in HF and they are used in the routine clinical practice for biochemical diagnosis of HF. The plasma concentration of natriuretic peptides (NPs) can be used as a clinical diagnostic test, especially in non-acute conditions *(Kim H.N.).* The increased natriuretic peptides help in the initial diagnose, identifying these subjects who need more cardiac tests; patients with values under lower limit border exclusion of significant cardiac dysfunction don't need echocardiography. Patients with normal plasma concentration of NP most likely are without HF. The use of natriuretic peptides is recommended for exclusion of HF, but not for conformation of this diagnose. There are many cardiovascular and non-cardiovascular reasons for increase of natriuretic peptides, which can decrease their diagnostic value in HF. AF, age and kidney failure are major factors make difficult the interpretation of results from the NP examination. Values of NP can be disproportionally lower in patients with obesity *(Madamanchi C) (Maisel A).*

We did not find significant relationship between values of biomarkers NT-proBNP, IL-18 and the indicators LV EF and NYHA F.C. The relationship between plasma levels of BNP and NT-proBNP and the risk of cardiovascular complications in patients with CHF has been reported in literature to date, but it is not clear whether changes in serum levels of BNP and NT-proBNP are predictors of higher morbidity in patients with CHF. *Savarese G* et al., analyzed results from 19 clinical trials, with 12891 patients with HF with mean follow-up 9,5 months after dehospitalization. The aim was to clarify the relationship BNP and NT-proBNP plasma levels and the risk for rehospitalizations because of HF worsening. The active treatment and the reduction of BNP and NT-proBNP significantly decrease the hospital stay in the hospitalized patients with ECCHF. Authors used meta-regression analysis and demonstrate that BNP and NT-proBNP reduction during the hospital stay decreases risk of hospital treatment for HF worsening. Analyzing the results from our study we did not find statistically significant relationship between NT-proBNP values, the frequency of rehospitalizations and mortality during the 24 months follow-up (p>0,005), but in patients with higher values of NT-proBNP higher number of rehospitalizations for CHF worsening during the period of follow-up is registered. Data from scientific literature shows that NT-proBNP and BNP plasma levels are not always increasing proportionally in patients with HF. Wang Y et al., simultaneously assess NT-proBNP and BNP levels in 1464 patients hospitalized for ECCHF were followed-up for 533 days with the aim to investigate whether parallel examination of NT-proBNP and BNP have additional prognostic value in HF. According to the authors NP-proBNP and BNP plasma levels are not always increased proportionally in patients with HF and a combination of NT-proBNP and BNP examination can increase the prognostic value of the natriuretic peptides in regard to the risk of unfavorable events in the hospitalized HF patients.

HF is often accompanied by reduction of the glomerular filtration. The frequency of the reduced glomerular filtration in patients with HF is observed in 30-60%. Different important interactions between the cardiac and renal disease exist. The mortality in patients with HF is higher in the patients with increased creatinine levels and with reduced glomerular filtration, respectively. The worsening of the renal function in patients with HF is defined as reduction of the glomerular filtration as the most used test for its measuring is serum creatinine levels. Another alternative of serum creatinine levels determination of serum Cystatin C levels. Cystatin C is defined as "ideal renal marker".

In our study Cystatin C was examined in 78 patients. 89.7% from the studied participants showed increased Cystatin C levels. Patients with values of eGFR Cystatin C values in the interval 30-60 were the highest relative proportion (43.6%), followed by these subjects with 60-90 (29.5%) and the smallest number of patients are over 90 – 7.7%. There is statistically significant relationship between values of biomarkers NT-proBNP, Cystatin C and eGFR Cystatin C. The correlation of eGFR Cystatin C with the considered markers is inversely proportional, strong with NT-proBNP ($p<0,001$) and very strong with Cystatin C ($p<0,05$). The type of relationship with NT-proBNP is linear.

In patients with higher values of NT-proBNP – marker of advanced HF, the number of the subjects with eGFR Cystatin C > 30 - < 60 ml/min 1,73 m2 as well with eGFR Cystatin C ≥ 60 - < 90 ml/min 1,73 m2 is statistically significantly higher which confirms the thesis that with the advance of heart failure early renal injury occurs. This is a predisposition for development of cardiorenal syndrome. Our results also confirm the thesis that eGFR Cystatin C is more accurate marker for assessment the stage of the renal injury as well as recent data from literature that the predictive value of Cystatin C and eGFR Cystatin C increases with parallel examination of NT-proBNP. According to *Dupont M* et al., the renal function is strong predictor for unfavorable events in HF and Cystatin C is promising and better marker from creatinine for glomerular filtration. After correction of the traditional RF and NT-proBNP Cystatin C remains independent predictor ($p<0,001$) of major cardiovascular events in CHF. The examination of Cystatin C and eGFR Cystatin C has significant predictive value especially in patients with relatively preserved renal function (237). Cystatin C has additive prognostic value towards BNP. In patients in the highest tercile of both indicators have 45% mortality risk, MI and ischemic stroke ($p<0,0001$) in comparison with 12% risk when the two variables are the lowest tertile.

The increase in the frequency and the constantly high CHF morbidity and mortality rate suggest that RF remain unaffected from the current HF treatment options. According to the existing data an activation of the immune system in CHF occurs. This proves the role of inflammation in CHF as probably the inflammation role is more complex and also it can have an adaptive and cardioprotective effects. There is evidence that the proinflammatory cytokines as TNF-α, IL-1β, IL-6 take part in the pathogenesis of the advanced HF. Cytokines have cardial depressive effect, promote systemic catabolism, cardiac hypertrophy and myocytes' apoptosis in CHF *(Yndestat T).*

We found significant relationship between the quantitative values of biomarkers NT-proBNP, IL-18 and Cystatin C and rehospitalizations for HF. The available data are statistically representative only for the rehospitalizations at 6th month. The mean value of IL-18 in the rehospitalized patients for this period is significantly higher than the non-rehospitalized. In order to assess qualitatively the relationship of the examined biomarkers with the risk for rehospitalization at 6th month we used binary logistic regression. Our results from ROC curves for determination of cut-off point in delimitation of the rehospitalized from the non-rehospitalized patients at 6th month are: a) NT-proBNP (area under curve 0,511, p=0,917), b) IL-18 (area under curve 0,708, p=0,044), c) Cystatin C (area under curve 0,572, p=0,484).

The biomarker IL-18 was the only one with statistically significant odds ratio in analysis by the determined cut-off point from ROC curve. This assumes that patients with value of IL-18 ≥ 219 have approximately 6 times higher risk for rehospitalization until the 6th month compared with the patients with lower values. IL-18 < 219 has extremely high negative predictive value for rehospitalizations at 6th month.

Cytokines have cardiodepressive effects and promote myocyte apoptosis in CHF. IL-18 has been suggested to take part in the HF pathogenesis contributing for the immune activation and cardiac dysfunction in CHF. *Naito Y* et al., for the first time prove that IL-18 serum

levels are increased in patients with CHF. Moreover its higher levels correlate with lower LV EF and with higher concentration of TNF-α. Serum IL-18 levels are significantly higher in patients with CHF in comparison with patients without CHF (255 (30) vs. 83 (9) pg/ml, p<0,001, respectively). IL-18 plays role in the pathogenesis of CHF and its specific functions are object of investigations. TNF-α serum concentration is also higher in patients with CHF in comparison with patients without symptoms of HF (1,9 (0,3) vs. 1,1 (0,1) pg/ml, p<0,05). The results of our study confirm that IL-18 values are increased in the hospitalized patients with ECCHF. **Our contribution is in defining of a cut-off point for IL-18 (219 pg/ml) over which the frequency of rehospitalizations for ECCHF during the first six months significantly increases. The examination of IL-18 in the hospitalized patients with ECCHF would contribute for more accurate stratification of the risk for rehospitalizations.** According to *Raeburn CD* it is possible IL-18 to mediate via induction of and/or synergistically with IL-1β, ICAM and VCAM-1, endotoxic myocardial dysfunction

The total number of dead patients is 45 or 13.7% from the examined contingent by us. 28 (62.2%) were men and 17 (37.8%) were women. The proportion of dead patients because of CVD reasons was significantly higher – 88.9% vs. subjects died because of other reasons – 11.1%. When we analyzed the causes of death in the hospitalized patients with HF we found that the most common cause for exitus in patients with HF is sudden cardiac death (SCD) – 42.5%. These data confirm publication of *Diseroti M* who concludes that SCD is very common in patients with heart failure especially with NYHA II and III F.C. (52-80% and 30-50% of all deaths, respectively). The mechanism of SCD depends on data whether HF is secondary as a consequence from IHD or other disease. SCD in IHD is mainly result from ventricular arrhythmias (VT/VF), which are caused by reentry mechanism in the infarct zone or acute ischemic episodes or bradyarrhythmia. Bradycardia and pauses are often observed in patients with HF. The pauses are associated with worse prognosis in patients with CAD and left ventricular dysfunction as bradyarrhythmia can have important contribution for sudden cardiac death in HF.

When we analyzed the survival in our study we did not find statistically significant relationship between gender, age, obesity and cardiovascular mortality. The mortality in men and women is comparable, but between 9th and 12th month sharper decline of survival in men is observed. We found that ejection fraction < 40% is significant factor for higher mortality during the two years follow-up period. Patients with reduced EF < 40% have significantly lowest mean survival, whereas patients with preserved EF ≥ 50 and diastolic dysfunction have statistically significantly highest survival. *Chang RK* et al., reported similar results. The authors followed-up patients from Get With The Guidelines – HF registry and concluded that patients with HFrEF have slightly higher mortality in comparison with patients with HFmrEF and HFpEF.

The total survival in the 328 hospitalized followed-up patients, from who 45 died is 13.7%. The highest mortality is observed during the first 6 months – 15 cases or 1/3 from the total number of dead patients for the period of follow-up (45 patients). The second mortality rank was taken by the second six months, when 14 (31.1%) of the exitus cases occurred. The minimally registered survival was one week and the maximal – 2 years, the six months survival is 95.4%, one year – 91.2%, 18-months -89.3% and two years survival – 86.3%. The cumulative survival falls faster during the first year of follow-up, than the declining cumulative survival becomes slightly smoother and it is 0,85 at 24th month.

After a statistical processing of our results the risk profile of hospitalized patients with ECCHF was defined, and three groups were formed according to the degree of risk for rehospitalizations and death:

- ***Low risk group*** (patients with: smaller number of non-cardiac and cardiac comorbidities; sinus rhythm; without rhythm and conduction abnormalities; IL-18 < 219 pg/ml; HFpEF – EF ≥ 50%; diastolic left ventricular dysfunction);

- *Intermediate risk group* (patients with: HFmrEF; EF 40—49%; sinus bradycardia; COPD; anemia and patients with smaller number of cardiac comorbidities and with uncomplicated clinical course of HF during the time of hospitalization);
- ***High risk group*** (patients with: ischemic heart disease; previous myocardial infarction; type II diabetes mellitus with duration more than 5 years; gout; atrial fibrillation; left bundle branch block; AV block I degree; increased IL-18 $\geq$ 219 pg/ml; HFrEF – EF < 40% and clinical course of HF during the hospitalization (pleural effusion, pericardial effusion, ascites).

The examination of IL-18 in the hospitalized patients with ECCHF could contribute for more accurate risk stratification for rehospitalizations. The clinical practice application of the formed risk stratification scale in hospitalized patients with ECCHF is a predisposition for differentiated diagnostic and therapeutic approach during the time of hospitalization. It will be useful the high risk patients after dehospitalization and regarding the ambulatory therapy to be more strictly monitored and controlled.

VII. CONCLUSIONS:

1. Subjects with HF are polymorbid (more than half of the hospitalized patients with HF are with three and more concomitant cardiac diseases). IHD and AH are leading reasons for CHF in the examined contingent.

2. Patients with CHF are with high percentage of non-cardiac comorbidities (25.3% have two and 23.2% have three concomitant non-cardiac diseases). The most frequent non-cardiac comorbidities in patients with HF are: T2DM, COPD, anemia and gout.

3. Regarding the non-cardiac comorbidity in our contingent with HFpEF, we estimated statistically significant difference between men and women in the frequency of the thyroid pathology, anemia and CKF. The anemia ($p<0,001$) and the thyroid pathology ($p=0,01$) are more frequent in women, whereas patients with CKF ($p=0,003$) predominate in men.

4. The relative proportion of the survived patients at 12th and 24th month is significantly higher in patients without cardiac comorbidity. The mortality is significantly higher until the end of the second year in patients with HF with chronic IHD and previous MI and with patients with AF before hospitalization as well.

5. The mortality until 12th month is significantly higher in patients with HF and type II Diabetes mellitus with duration more than 5 years and in patients with hyperuricemia and gout.

6. We found a significant difference between rehospitalizations for HF at 3rd month and type of the conduction abnormalities, LV EF and left ventricular hypertrophy, the rehospitalized patients have significantly higher relative proportion of LBBB ($p=0,002$), reduced EF < 40% ($p=0,002$) and without data for left ventricular hypertrophy ($p=0,04$).

7. A significant relationship between rehospitalizations for HF at 24th month and clinical characteristics of hospitalized patients for ECCHF was found with other complications (pleural effusion, $p=0,042$) the type of conduction abnormalities and LV EF (reduced EF < 40%, $p=0,002$).

8. There is no statistically significant relationship between the included in the study cardiac and non-cardiac comorbidities, and the frequency of rehospitalizations for ECCHF for the period of follow-up.

9. There is no statistically significant relationship between the indicators' values of biomarkers: NT-proBNP, IL-18, and LV EF, and NYHA F.C. as well. The serum levels of Cystatin C and eGFR $_{MDRD}$ Cystatin C are more accurate markers for assessment of the renal injury stage, as the predicting value of Cystatin C and eGFR Cystatin C regarding the course of HF is increased with parallel examination of NT-proBNP.

10. A significant association between the quantitative values of IL-18 and the frequency of the rehospitalizations for HF until 6th month was found.

11. A cut-off point of IL-18 (219 pg/ml) was defined. The frequency of rehospitalizations for ECCHF during the first sixth months significantly increases up to six times with IL-18 values > 219 pg/ml. Values of IL-18 < 219 pg/ml are with high negative predictive properties regarding rehospitalizations for ECCHF for six months period.

VIII. CONTRIBUTIONS:

CONFIRMATIVE CONTRIBUTIONS

1. Patients with HF are polymorbid most frequently with three and more cardiac comorbidities. The ischemic heart disease and arterial hypertension are leading causes for development of chronic heart failure.

2. The percentage of non-cardiac comorbidities is high in patients with chronic heart failure. The most frequent non-cardiac comorbidities and conditions in patients with HF are: type II diabetes mellitus, chronic obstructive pulmonary disease, anemia, hyperuricemia and gout.

3. There is weak correlation between the measures left ventricular ejection fraction and NYHA F.C.

4. There is no significant relationship between the values of biomarkers NT-proBNP, IL-18 and the indicators LV EF and NYHA Functional Class.

5. Serum levels of the cytokine IL-18 are increased in ECCHF.

6. Serum levels of Cystatin C and eGFR MDRD Cystatin C are more accurate markers for renal injury in the course of CHF.

7. The mortality in patients with HF and chronic IHD and previous MI is significantly higher. The mortality in patients with HF and permanent atrial fibrillation is also significantly higher.

8. The mortality until 12th month is significantly higher in patients with HF and T2DM

9. Patients with HF and hyperuricemia and gout are with significantly higher mortality until the end of the first year.

10. The most frequent cause for death in patients with HF is sudden cardiac death.

ORIGINAL CONTRIBUTIONS

1. Complex parallel examination of three markers: NT-proBNP, Cystatin C, IL-18 in hospitalized patients with ECCHF was performed.

2. Serum levels of Cystatin C with calculated eGFR MDRD Cystatin C in patients with exacerbated chronic heart failure was investigated for the first time in Bulgaria.

3. Comparison of Cystatin C, $eGFR_{MDRD}$ Cystatin C and creatinine and $eGFR_{MDRD}$ creatinine was performed for assessment of renal injury in the course of CHF.

4. IL-18 as an inflammatory marker and marker for cardiomyocytes' apoptosis in patients with CHF was investigated for the first time in Bulgaria.

5. It was determined for the first time in Bulgaria that serum levels of the cytokine IL-18 in ECCHF are increased.

6. The definition of IL-18 cut-off point (219 pg/ml) is our own original contribution. The frequency of rehospitalizations for ECCHF during the first six months significantly increases (until six times) if IL-18 values are over 219 pg/ml. Proving of high negative predictive values of IL-18 < 219 pg/ml regarding rehospitalizations for ECCHF for six month period is another original contribution.

A. PUBLICATIONS RELATED TO THE DISSERTATION:

1. Nikolov AG, Tzekova ML, Kostov KM, Blazhev AB. ASSOCIATION BETWEEN SERUM MATRIX METALLOPROTEINASE-12/ANGIOTENSIN II PROFILE AND LEFT VENTRICULAR HYPERTROPHY IN PATIENTS WITH HEART FAILURE. Archives of the Balkan Medical Union 2020; 55(2): 233-242; ISSN PRINT 1584-9244; ISSN ONLINE 2558-815X **(SJR- 0.218)**

2. Nikolov AG, Blazhev AB, Tzekova ML, Kostov KM, Popovski NK. Serum Levels of Antibodies to Advanced Glycation End Products in Patients with Type 2 Diabetes Mellitus and Hypertension. Folia Medica 2020; 62(2): 295-301; ISSN 1314-2143 (online) | ISSN 0204-8043 (print) **(SJR- 0.252)**

3. Nikolov AG, Tzekova ML, Kostov KM, SERUM BIOMARKERS OF COLLAGEN TYPE I AND TYPE III TURNOVER IN HEART FAILURE – THE NEED FOR REAPPRAISAL. Acta Medica Croatica, 2020; 74(2): 145-153; ISSN PRINT 1330-0164; ISSN ONLINE 1848-8897 **(SJR- 0.116)**

4. Kostov K., Tzekova M., Nikolov A. Clinical significance of biomarkers: NT-ProBNP, Cystatin C and IL-18 in patientswith chronic congestive heart failure. Medical Magazine. 2018, 10, 22-24, ISSN: 1314-9709 .

5. Nikolov A., Tzekova M., Kostov K. Novel cardiac biomarkers in heart failure - from theory to practical aplication. Medical Magazine. 2018, 10,18-21, ISSN: 1314-9709.

6. M. Tzekova , Kostov K . Algorythm for diagnosis of Acute heart failure. Recommendations of European Society of Cardiology. MEDINFO, 2017, 7, 6-9, ISSN:1314-0345.

7. Nikolov A., Kostov K., Tzekova M. Serum biomarkers of cardiac extracellular matrix (cardiac collagen turnover) in patients with essential hypertension and heart failure with mid-range ejection fraction. Medical Magazine, 74, 03.2020, 72-77, ISSN: 1314-9709

B. LIST OF PARTICIPATIONS IN CONGRESSES ABROAD

1. Nikolov A., Tsekova M, Kostov K., Blazhev A., Lozanov L., Drenovski T. Serum levels of anti-collagen type IV IgG antibodies are associated with high risk of atherosclerosis in diabetic patients with essential hypertension. 25TH ISCOMS (International Student Congress of (bio)Medical Sciences), 04-08 June, 2019 Amsterdam, The Netherlands.

2. Abstract 591- Association between serum anti-elastin IgA antibodies levels and hypertension treatment and control in diabetic patients. A. Nikolov, M. Tzekova, K. Kostov, A. Blazhev, N. Popovski. European Atherosclerosis Society (EAS) 2020 Virtual Congress, 04-07 October 2020, poster, Abstract in print Atherosclerosis e-Supplements; ISSN: 1567-5688 (IF-3.968).

3. Abstract 1597- Circulating serum markers of collagen type III synthesis in high atherogenic risk patients with heart failure and coronary artery disease. A. Nikolov, M. Tzekova, K. Kostov, A. Blazhev, N. Popovski. European Atherosclerosis Society (EAS) 2020 Virtual Congress, 04-07 October 2020 , poster, Abstract in print Atherosclerosis e-Supplements; ISSN: 1567-5688 (IF-3.968).

C. LIST OF PARTICIPATIONS IN CONGRESSES IN BULGARIA

4. **Abstract**. Atanasova GL., Tzekova ML., Kostov KM., Stefanova P., Valeva I.detection of cognitive impairment in metabolic syndrome. XVII International medical scientific conference for students and young doctors. Medical University Pleven, Bulgaria, 20.10.- 02.11.2019. Abstract book. 2019:62.

5. Asparuh G. Nikolov, Maria L. Tzekova, Alexander M. Blazhev, Konstantin M. Kostov, Nikola K. Popovski, Teodor V. Drenovski. Serum Levels of Angiotensin II and Tissue Inhibitor of Matrix Metalloproteinase-3 in Patients with Heart Failure and Essential Hypertension. Journal of Biomedical and Clinical Research. Vol.12 Number 1, Supplement 2, 2019, 39 (ISSN: 1313-9053)