

The importance of apoptotic activity and plasma NT-proBNP levels in patients with acute exacerbation of decompensated heart failure and their relation to different drugs and comorbidities

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Abstract

Objective: To demonstrate the presence and importance of apoptotic activity in heart failure during acute exacerbations and to investigate the effects of different drugs used and co-morbidities on levels of N-Terminal pro-Brain Natriuretic Peptide and apoptotic activity on admission and during hospitalisation.

Methods: The descriptive study was conducted at the emergency department of Istanbul University Cardiology Institute between October 2010 and May 2011 and comprised patients with complaints of shortness of breath, and who were evaluated as acutely exacerbated decompensated heart failure with an aetiology of ischaemic or dilated cardiomyopathy. Apoptotic activity and N-Terminal pro-Brain Natriuretic Peptide levels were measured on admission and on the seventh day of treatment. SPSS 15 was used for statistical analysis.

Results: Of the 89 patients in the study, 67(75%) were males. Overall mean age of the study sample was 61 ± 12 years. Patients who had N-Terminal pro-Brain Natriuretic Peptide levels higher than 6000pg/ml on admission had greater in-patient mortality rate ($p < 0.001$). N-Terminal pro-Brain Natriuretic Peptide levels decreased significantly on the seventh day of treatment compared to the admission values ($p < 0.012$). Apoptotic activity levels, although not statistically significant, increased on the seventh day compared with admission values ($p < 0.12$). Apoptotic activity levels on the 7th day were associated with in-patient deaths ($p < 0.002$). Dopamine infusion in the treatment group during hospitalisation significantly increased apoptotic activity ($p < 0.035$), whereas there was a trend towards decreased apoptotic activity levels with spironolactone ($p < 0.07$). Treatment with beta-blockers did not change apoptotic activity levels ($p < 0.751$), whereas lack of beta-blocker therapy increased apoptotic activity ($p < 0.02$).

Conclusion: N-Terminal pro-Brain Natriuretic Peptide may be an important risk predictor in decompensated heart failure exacerbations during hospitalisation but not apoptotic activity. Beta-blocker therapy seems to positively affect the process of apoptosis.

Keywords: Apoptotic activity, NT-proBNP levels, Decompensated heart, Comorbidity. (JPMA 64: 884; 2014)

Introduction

Patients with acute exacerbation of heart failure demonstrate higher risk with respect to death and re-hospitalisation rates when compared with cases of chronic but stable disease. Major prognostic biomarkers used for in-patients with heart failure are troponin, C-Reactive Protein (CRP) and natriuretic peptides. Natriuretic peptide test is most widely used to confirm the diagnosis of acute decompensated heart failure in patients presenting with acute symptoms. Furthermore its importance in predicting prognosis, and guiding the treatment has been demonstrated in numerous studies.¹⁻⁹

The process extending from myocardial remodelling and compensated hypertrophy to heart failure encompasses complex molecular and cellular events, growth and

hypertrophy of myocytes, re-expression of foetal gene programmes, decrease in the expression of adult gene programme, and death of myocytes because of necrosis and apoptosis. Apoptosis has been demonstrated in myocardial infarction (MI), dilated cardiomyopathy, end-stage heart failure, and in myocardial tissue samples obtained during ischaemia/reperfusion damage induced during animal experiments. Since inhibition of apoptosis is cardioprotective in many conditions, this process is the potential target of therapeutic interventions aiming at the prevention of heart failure.¹⁰⁻¹⁴

The current study was designed to demonstrate both the presence of apoptotic activity (which is known to be effective in advanced stages of heart failure) in the acute exacerbations of heart failure, and also its correlation with N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) levels, if any. We also planned to determine the interactions between admission and the seventh day NT-proBNP values, and apoptotic activity levels of patients presenting with symptoms of decompensated heart

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failure, and also the inter-relationship of these levels with in-patient mortality rates during the hospitalisation period.

Patients and Methods

The descriptive study was conducted at the emergency department of Istanbul University Cardiology Institute between October 2010 and May 2011 and comprised patients with complaints of shortness of breath, and who were evaluated as acutely exacerbated decompensated heart failure with an aetiology of ischaemic or dilated cardiomyopathy. All drugs used on the patients and comorbidities were registered on admission and in hospitalisation period.

The sample size was calculated by the formula: $n = Z^{1-\alpha} \cdot S^2 / d^2$ ($n = 86 = 1.96^2 \times (1 - 0.55/2) \times 0.50^2 / 0.05^2$) (N =Population Size; n = Sample Size; S = Standard Error; 0.05 (5%) (Confidence Interval [CI] 95%); Confidence Interval: upper=95%; Lower: 5%; The Z-values for confidence levels were: 1.645 = 90 percent confidence level; 1.96 = 95 percent confidence level; 2.576 = 99 percent confidence level (Z for $p=0.05, 0.01, 0.001$ are 1.96, 2.58 and 3.28 Z values respectively); d = Relative Standard Error) and it was confirmed by automatic sample size calculator. The required sample size was 89.

After approval of the study protocol by the institutional ethics committee, informed written consent was obtained from all the subjects. The study was performed in compliance with the ethical standards laid down in the 1975 Declaration of Helsinki.

For the purpose of the study, the term 'heart failure' was used to describe the symptomatic syndrome, graded according to the New York Heart Association (NYHA) functional classification.⁷

Patients with the following characteristics were excluded from the study: presence of heart failure with concomitant renal failure (serum creatinine >2.0 mg/dl or Glomerular filtration rate (GFR) <30 ml/min/ 1.73 m²), primary pulmonary hypertension, acute coronary syndrome within the preceding two months, isolated right heart failure (RHF), heart failure due to congenital heart disease, advanced valvular disease (aortic and/or mitral valvular insufficiency), age <18 years, life expectancy of <1 year because of non-cardiac aetiologies (i.e. malignancies).

After enrolment and before initiation of the drug therapy, two 10-ml blood samples were drawn from the antecubital vein, and placed into an ethylenediaminetetraacetic acid (EDTA) tube for the

measurement of NT-proBNP and also into a biochemical test tube for the analysis of plasma apoptosis. The samples were centrifuged at $+4^\circ\text{C}$ and at 1500rpm for 5 minutes. Upper phase plasma supernatant was taken into another tube for the measurement of NT-proBNP and analysis of apoptosis. All reagents required for the study were prepared according to the instructions of the producer.

Regression, and deterioration of patients' symptoms were noted, and their NYHA functional status was assessed. Blood samples were drawn again on the seventh day to constitute the control group which was formed within the same patients, including those who died after the first seven days of hospitalisation.

Quantitative analysis of NT-proBNP was performed using chemiluminescence immunoassay method (IMMULITE-1000 model SIEMENS). For the Analysis of apoptosis, enzyme-linked immunosorbent assay (ELISA) method using Cell Death Detection kit (ELISApplus; Roche Diagnostics; Cat no. 11774425001, USA) was used.

All data was collected on admission and on the 7th day of hospitalisation period. All the variables were entered into SPSS 15 for statistical analysis. Concordance of variables used in the study to the normal distribution profile was analysed with Kolmogorov-Smirnov Z test. Descriptive statistics were computed and presented as means and standard deviations (SD) for continuous variables like age, and Left Ventricular Ejection Fraction (LVEF). Frequencies and percentages were computed for gender, diabetes, hypertension, drugs used, their frequencies of usage, and related comorbidities. Distribution of qualitative data was expressed as percentages. Data within normal distribution curve were defined as mean $\pm$ standard deviation ($X \pm SD$). Data demonstrating a skewed distribution were expressed as medians (minimal and maximal value). The latter data was analysed using non-parametric tests, and for normally distributed data, parametric tests were used. For normality analyses of data, intergroup independent student t-test, and for the comparison of the means of two dependent groups, paired samples t-test was used. For intergroup comparisons of numeric variables with skewed distribution Mann Whitney U test was employed. For variance analyses of repeated measurements of multiple dependent variables, Generalised Linear Model statistics were used. Distribution of qualitative data was expressed as percentages. For suitable data chi-square test was used to determine statistical significance. Pearson's linear correlation analysis was used for correlation values between the 7th day apoptotic activity and ejection fraction (EF), Left Ventricular Dysfunction (LVd), and

admission NT-proBNP versus CRP (r values: -1 to +1). Multivariate logistic regression analysis was performed to determine potential influential factors on the decline in the levels of apoptotic markers. Level of significance was set at $p \leq 0.05$.

Results

Of the 89 patients in the study, 67(75%) were males. Overall mean age of the sample was 61 ± 12 years. Of the total, 33(37%) patients were diagnosed as idiopathic dilated cardiomyopathy and 56(63%) as ischaemic cardiomyopathy.

Table-1: Demographic, clinical characteristics, laboratory values, echocardiographic parameters and medications used.

Characteristics	Values	Characteristics	Values
Gender (M)	67 (75%)	Creatinine (mg/dL)	1.2 ± 0.3
Age (years)	61 ± 12	GFR (ml/min/1.73m ²)	65 ± 17.6
Hypertension (n, %)	54 (61%)	Glycaemia (mg/dL)	139 ± 70.2
Smokers (n, %)	44 (49%)	HbA1c (%)	7.7 ± 1.5
Diabetes mellitus (n, %)	42 (47%)	Hct (%)	38 ± 4.0
Hyperlipidaemia (n, %)	14 (16%)	Na (mEq/L)	134 ± 4.0
Previous MI (n, %)	54 (60%)	K (mEq/L)	4.3 ± 0.5
Previous CABG/PTCA (n, %)	34 (38%)	WBC /mm ³	8550 ± 2489
Dilated CMP (n, %)	33 (37%)	CRP (mg/L)	48 ± 56.2
Ishaemic CMP (n, %)	56 (63%)	CK (U/L)	183.1 ± 211.0
Killip class 3 (n, %)	62 (70%)	AST (U/L)	29.3 ± 14.9
Killip class 4 (n, %)	27 (30%)	ALT (U/L)	33.5 ± 26.5
AF (n, %)	14 (16%)	TSH (milliU/L)	3.7 ± 3.4
Pacemaker / ICD /			
CRT implanted (n, %)	2 (2%)	Uric acid (mg/dL)	7.6 ± 2.5
SBP (mm Hg)	118 ± 18.5		
Heart rate (bpm)	83 ± 20.6		
Acetylsalicylic acid	65 (73%)	LVEF (%)	$0.30.0 \pm 8.3$
Beta- blockers	45 (51%)	LVs (cm)	5.52 ± 1.02
ACE-I	37 (42%)	LVd (cm)	6.2 ± 0.98
ARB	7 (8%)	LA (cm)	4.81 ± 0.82
Statins	34 (38%)	IVS (cm)	1.0 ± 0.32
Furosemide	86 (97%)	RV (cm)	2.8 ± 0.71
Spironolactone	61 (69%)	PAP (mmHg)	38.8 ± 7.9
Warfarin	20 (23%)		
Dopamine perfusion			
(during hospitalization)	31 (35%)		
Dobutamine perfusion			
(during hospitalization)	21 (24%)		
Nitrates perfusion	29 (33%)		

PTCA: Percutaneous transluminal coronary angioplasty. MI: Myocardial infarction. AF: Atrial fibrillation. CABG: Coronary artery bypass graft. CMP: Cardiomyopathy. SBP: Systolic blood pressure. LVd: Left ventricle diastolic diameter. LVs: Left ventricle systolic. IVS: Interventricular septum. LA: Left atrium. LVEF: Left ventricular ejection fraction. RV: Right ventricle. PAP: Pulmonary arterial pressure. GFR: Glomerular filtration rate. ACEI: Angiotensin-converting-enzyme inhibitor. ARBs: Angiotensin II receptor blockers. ICD: implantable cardioverter defibrillators. CRT: Cardiac resynchronisation therapy. CK: Creatine Kinase. Hct: Haematocrit. Na: Sodium. K: Potassium. WBC: White blood cells. AST: Aspartate aminotransferase. ALT: Alanine transaminase. TSH: Thyroid-stimulating hormone. HbA1c: Glycated haemoglobin

Table-2: Comparison between admission, and 7th day NT-proBNP values with clinical and laboratory parameters, comorbidities and medications used during hospitalisation.

Characteristics	Admission NT-proBNP (pg/mL) (n=89)	Seventh day NT-proBNP (pg/mL) (n=89)	p-value
All patients	6667 ± 5494.4	4652 ± 4345.6	0.012
Killip class 3 on admission (n= 62)	7231.6 ± 970.6	4362.6 ± 704.5	0.007
Killip class 4 on admission (n= 27)	5397.7 ± 1146.4	5304.2 ± 1168.7	0.925
Compensated patients (n= 82)	6273.7 ± 5427.9	4108.8 ± 4060.2	0.012
Deceased patients (n= 8)	11391.0 ± 4403.8	11174.5 ± 1144.2	0.906
Gender; male (n=67)	66964.9 ± 2810.3	3846.2 ± 4026.0	0.011
Gender; female (n=22)	6964.9 ± 2810.3	6640.7 ± 4599.6	0.729
GFR > 60 ml/min/1.73m ² (n=52)	5727.5 ± 1012.5	4587.6 ± 810.9	0.053
GFR 30–60 (n=37)	5193.4 ± 1161.3	4034.9 ± 902.2	0.11
Patients with DM (n=42)	7733.2 ± 5990.7	3916.7 ± 779.9	0.02
Patients without diabetes (n=47)	5885.7 ± 5060.3	5191.8 ± 4775.8	0.33
Patients with HT (n=54)	6746.4 ± 5822.5	3910.5 ± 3161.5	0.018
Patients without hypertension (n=35)	6540.8 ± 5068.5	5839.2 ± 5654.2	0.423
Fasting blood glucose > 120 mg/dl on admission (n=39)	6684.9 ± 6491.7	1958.5 ± 1252.2	0.009
Fasting blood glucose <120mg/dl (n=48)	6107.9 ± 4567.7	5827.4 ± 4635.8	0.694
Ischaemic CMP (n=56)	5690.6 ± 5336.2	3869.5 ± 3835.4	0.098
Dilated CMP (n=33)	7898.9 ± 5558.5	5639.4 ± 4819.5	0.065
ASA users (n= 65)	6606.2 ± 4311.7	4916.3 ± 3983.7	0.04
ASA non-users (n=24)	6765.2 ± 7119.1	4229.9 ± 4948.7	0.205
Beta- blocker users (n=45)	7804.7 ± 4069.9	6462.1 ± 4429.9	0.045
Beta-blocker non-users (n=44)	4988.4 ± 6867.9	1980.7 ± 2479.1	0.088
ACE-I users (n= 37)	6025.4 ± 6023.7	4054.7 ± 3672.6	0.132
ACE-I non-users (n=52)	7138.1 ± 5125.6	5090.5 ± 4792.9	0.51
Sprinolactone users (n=61)	6585.9 ± 5312.9	5115.3 ± 4643.9	0.083
Sprinolactone non-users (n=28)	6938.5 ± 6307.0	4109.0 ± 3777.1	0.092
Statin users (n=34)	5132.1 ± 3578.3	4978.1 ± 2552.1	0.146
Statin non- users (n=55)	7626.8 ± 6272.6	5698.7 ± 4912.8	0.119
Dopamine users (n=31)	6559.6 ± 4813.7	6540.7 ± 4902.1	0.981
Dopamine non-users (n=58)	6775.1 ± 6195.8	2763.9 ± 2659.9	0.003
Patients with AF (n=42)	6157.0 ± 5666.9	4239.6 ± 4262.9	0.098
Patients with sinus rhythm (n=47)	7139.8 ± 5393.4	5034.4 ± 4466.6	0.068
COPD (n=8)	5036.6 ± 4945.9	5707.2 ± 6964.3	0.499

A statistically significant decrease was seen between admission and the seventh day NT-proBNP values. NT-proBNP values decreased significantly between admission and the seventh day in male patients, in patients presenting with Killip class 3, in patients with diabetes or hypertension and beta-blocker and Acetylsalicylic Acid (ASA) users.

NT-proBNP: N-Terminal pro-brain natriuretic peptide. GFR: Glomerular filtration rate. DM: Diabetes mellitus. HT: Hypertension. CMP: Cardiomyopathy. ASA: Acetylsalicylic acid. ACE-I: Angiotensin-converting-enzyme inhibitor. COPD: Chronic obstructive pulmonary disease.

Clinical and laboratory parameters determined on admission and during hospitalisation were noted (Table-1). Mean systolic blood pressure, diastolic blood pressure, and heart rate on admission were 118 ± 18.5 mmHg, 77 ± 11.4 mmHg and 83 ± 20.6 bpm respectively.

On transthoracic echocardiography all patients had

Table-3: Comparison between apoptosis values on admission and the 7th day and clinical, laboratory findings, comorbidities, and medications used during hospitalisation.

Characteristics	Admission Apoptosis value (Base pairs cells/ml) (n=89)	Seventh day Apoptosis value (Base pairs Cells/ml) (n=89)	p-value
All patients	7.8339 ± 9.86678	10.7906 ± 14.03789bp/ml	0.126
Killip class 4 on admission (n= 27)	3.7975±3.0712	13.4033±17.1684	0.064
Compensated patients (n= 82)	8.0781±10.1151	11.2820±14.3522	0.118
Deceased patients (n= 7)	2.0930±1.0823	4.4186±1.1274	0.002
Gender male (n=67*)	9.2627±11.9744	14.2689±15.5880	0.07
Gender female (n=22)	5.4388±4.5384	5.5411±9.2550	0.968
GFR > 60 ml/min/1.73m2 (n=52)	5.8036±5.6169	10.2776±12.6151	0.087
GFR 30–60 (n=37)	11.5217±14.5073	12.3061±16.8454	0.789
Patients with diabetes(n=42)	6.9880±6.4582	5.8538±6.4161	0.452
Patients without diabetes(n=47)	10.4827±9.1090	12.9955±11.0397	0.246
Patients with hypertension(n=54)	9.8924±12.0697	10.3790±13.7620	0.794
Patients without hypertension(n=35)	10.6790±10.9572	11.9378±10.9607	0.476
Fasting blood glucose > 120 mg/dl on admission (n=39)	5.9866±6.8392	9.6421±14.2267	0.355
Fasting blood glucose>200mg/dl (n=39)	5.9866±6.8392	9.6421±14.2267	0.355
Ischaemic CMP (n=56)	9.4741±12.3848	14.1953±15.7301	0.102
Dilated CMP (n=33)	5.3174±3.1606	6.1129±9.6656	0.736
ASA users (n= 65)	6.4302±6.0301	8.2978±11.6502	0.444
ASA non-users (n=24)	10.3791±14.6005	15.9162±17.0490	0.104
Beta- blocker users (n=45)	7.8923±5.6149	7.2554±8.2710	0.721
Beta-blocker non-users(n=44)	7.7128±15.2617	17.8172±19.4979	0.02
ACE-I users (n= 37)	10.3974±13.9762	16.2430±18.0560	0.134
ACE-I non-users (n=52)	5.8310±4.4715	6.9150±8.3470	0.548
Sprinolactone users (n=61)	8.7222±4.9170	6.9173±5.5466	0.07
Sprinolactone non-users (n=28)	4.8236±5.0350	4.5336±2.5028	0.877
Statin users (n=34)	13.0946±13.0224	10.5844±13.8642	0.252
Statin non- users(n=55)	13.7331±3.0757	12.8717±14.2751	0.316
Dopamine users (n=31)	6.9384±6.0257	14.2216±14.1046	0.035
Dopamine non-users (n=58)	8.8810±13.3098	7.1839±13.4447	0.115
Patients with AF (n=42)	10.1048±12.3596	12.8867±16.9905	0.378
Patients with sinus rhythm (n=47)	5.3548±5.7853	8.9409±10.1016	0.127
COPD (n=8)	6.2845±4.2168	2.2451±2.3232	0.115

Apoptotic activity levels on the seventh day were associated with in-patient deaths(p=0.002).

Beta-blocker therapy seems to positively inhibits process of apoptosis (p=0.02). Dopamine infusion in the treatment group during hospitalisation significantly increased apoptotic activity (p=0.0035) whereas there was a trend towards decreased apoptotic activity levels with spironolactone(p=0.07).Dopamine infusion can be harmful to the implementation of the unconscious. Contrary to our expectations, ACE, ARB, and statins have neutral effects on the apoptosis process.

GFR: Glomerular filtration rate. CMP: Cardiomyopathy. ASA: Acetylsalicylic acid. ACE-I: Angiotensin-converting-enzyme inhibitor. COPD: Chronic obstructive pulmonary disease.

dilated left hearts with a mean LVEF of 30±8.3%, left ventricular (LV) systolic 5.52±1.02 and LV diastolic 6.2±0.98. Drugs used, their frequencies of usage, and related comorbidities were also noted.

Mean hospital stay of the patients was 15±10.4 days. Clinical status of 82(92%) patients regressed to Killip class 2 on the 7th day of hospitalisation. Seven (8%) patients died during hospitalisation. Mean hospital stay of the 7 patients who died was 26±10.8 days.

NT-proBNP and apoptosis values on admission, and the 7th day were compared (Table-2).A statistically significant decrease was seen between admission and the 7th day NT-proBNP values (p=0.012). However, the increase

Table-4: Pearson's linear regression and correlation analysis.

	R	P
Apoptosis seventh day vs EF	-0.414	0.003
Apoptosis seventh day vs LVd	0.481	0.001
Admission NT-proBNP vs CRP	0.273	0.048

A statistically significant negative correlation was found between LVEF measured echocardiographically, and on the 7th day apoptosis values (r=-0.414; p=0.003). Correlation between the 7th day-apoptosis values, and LV diastolic diameter was also statistically significant (r=0.481; p=0.001). A statistically weak correlation existed between NT-proBNP and CRP values measured on admission, (r=0.273; p=0.048).

EF: Ejection fraction

LVd: Left ventricular diameter

NT-proBNP: N-terminal pro-brain natriuretic peptide

CRP: C-reactive protein.

Table-5. Relationships among beta-blockers, age, glycaemia, diabetes mellitus, hypertension, heart rate and apoptosis (Multivariate Logistic Regression Analysis).

CI	P	Odds ratio	95 %
Beta-blocker users or not	0.005	11.5	2.1
Age (yrs)	0.206	0.964	0.9
Glycaemia	0.199	0.990	0.9
DM	0.697	0.745	0.1
HT	0.260	2.419	0.5
Heart rate (bpm)	0.481	1.012	0.9

In multivariate logistic regression analysis performed to determine potential influential factors on the decline in the levels of apoptotic markers, only beta-blocker usage independently predicted decrease in the apoptosis levels (odds ratio 11.5-fold increase; 95% CI (2.1-63.3); $p=0.005$).

DM: Diabetes mellitus. HT: Hypertension.

observed in the apoptosis values was not significant ($p=0.126$). NT-proBNP values decreased significantly between admission and the 7th day in male patients, in patients presenting with Killip class 3, in patients with diabetes or hypertension, beta-blocker and Acetylsalicylic Acid (ASA) users (Table-3).

Patients whose apoptosis values persisted or decreased on the 7th day after admission were compared with respect to various parameters. There was no decrease in the 7th day apoptosis values in 54 (80.3%) men, and 4 (19.7%) women. Degree of decrease in apoptosis values was not altered in smokers, diabetics, patients with hypertension or hyperlipidaemia neither with usage of ASA, Angiotensin-converting-enzyme inhibitor (ACEI), furosemide, statins, and nitrates. However, a significant drop in apoptosis was not observed in patients using beta-blockers ($p=0.751$). Spironolactone treatment decreased apoptotic activity levels non-significantly ($p=0.07$) when compared with non-users. A statistically significant increase in apoptosis values was detected on the 7th day in patients on dopamine ($p=0.035$), and non-users of beta-blockers ($p=0.020$) during hospitalisation.

A statistically significant negative correlation was found between LVEF measured echo-cardiographically, and on the 7th day apoptosis values ($r=-0.414$; $p=0.003$). Correlation between the 7th day apoptosis values, and LV diastolic diameter was also statistically significant ($r=0.481$; $p=0.001$). A statistically weak correlation existed between NT-proBNP and CRP values measured on admission ($r=0.273$; $p=0.048$) (Table-4).

In multivariate logistic regression analysis performed to determine potential effective factors on the decline in the levels of apoptotic markers, only beta-blocker usage independently predicted decrease in the apoptosis levels (odds ratio 11.5-fold increase; 95% CI 2.1-63.3; $p=0.005$). Among parameters related to correlations between

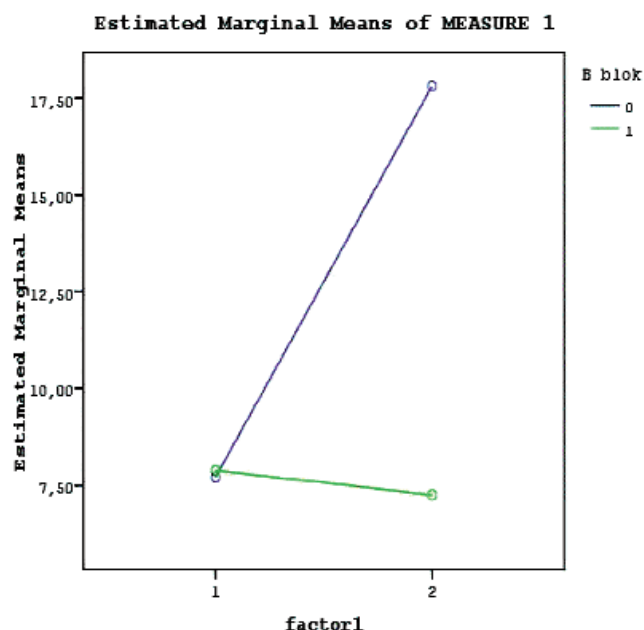


Figure: Apoptosis in patients using or not using beta-blockers; values (green line group of patients using beta-blockers, the blue line indicates the group not using beta-blockers).

laboratory parameters and decreasing or increasing apoptosis values, diastolic blood pressure and potassium values did not show any significant difference between patients with increased and decreased apoptosis values (Table-5, Figure).

Discussion

Acute exacerbation of decompensated heart failure demonstrates a higher risk of death, and re-hospitalisation when compared with chronic but stable patients. In-patient mortality displays a wide spectrum in various studies ranging from 2% to 20%.^{1,2} Clinical status of 82 (92%) patients included in our study improved to Killip class 2 on the 7th day of hospitalisation and treatment. Seven (8%) patients died during hospitalisation.

Natriuretic peptide tests are the most widely used analyses to confirm the diagnosis of acute decompensated heart failure in patients presenting with acute symptoms. Their significant roles in prognosis, treatment, and therapeutic decision-making processes have been demonstrated in various studies.^{7,8,15} In 90% of young, and healthy individuals, levels of BNP, and NT-proBNP have been detected to be <25pg/ml, and <70pg/ml, respectively. Current guidelines of heart failure have indicated that NT-proBNP is as valuable as natriuretic peptide BNP in diagnostic evaluations.⁷ It is used as a

prognostic marker, and its utility as a tool to monitor the response to treatment in acute heart failure has been advocated. Use of cut-off values of BNP ($<100\text{pg/ml}$), and NT-proBNP ($<300\text{pg/ml}$) has been recommended to discard the possibility of heart failure in patients who developed acute dyspnoeic episodes. Decreases in plasma concentrations of BNP and NT-proBNP have been observed secondary to many therapeutic modalities (diuretics, renin-angiotensin-aldosterone system [RAAS] blockade, beta-blocker, spironolactone, nesiritide) known to be beneficial in the treatment of heart failure among patients treated for acute decompensated heart failure. A significant and consistent decrease in NT-proBNP levels within 16 hours was observed in those with meaningful improvements in pulmonary capillary wedge pressure (PCWP) and in cardiac haemodynamic status.⁷⁻⁹

In 80% of our study participants, clinical improvement was observed on the 7th day of their hospital stay. A statistically significant decrease in NT-proBNP values was seen from admission up to the 7th day of hospitalisation ($p<0.012$). Still, in compliance with literature findings, a statistically significant drop from admission NT-proBNP values was detected on the 7th day of their hospitalisations in patients presenting with Killip class 3 heart failure, in males, diabetics with fasting blood glucose levels $>120\text{mg/dL}$, hypertensives, beta-blocker and ASA users ($p<0.007$; $p<0.011$; $p<0.009$; $p<0.018$; $p<0.045$; $p<0.040$ respectively). Patients who had NT-proBNP levels higher than 6000pg/ml on admission had greater in-patient mortality rate ($p<0.001$). Bettencourt et al. demonstrated predictive value of NT-proBNP concentrations measured on admission, and at discharge.⁸ Based on the results of their study, NT-proBNP $>6779\text{ng/l}$ was accepted as a criterion for re-hospitalisation and mortality risk. The study reported that a post-treatment NT-proBNP concentration of $<4137\text{ng/l}$ signified risk reduction, and indicated that each decrease of 1.000ng/l is associated with a 8% decrease in re-hospitalisation rates and mortality risk.⁸⁻¹⁵ Di Somma et al. had found a 58% decrease in NT-proBNP levels in patients with decompensated heart failure after a seven-day course of successful treatment. Differences in concentrations of NT-proBNP detected on admission, and at discharge indicate therapeutic success or risk of re-hospitalisation. Achievement of $>30\%$ drop in NT-proBNP indicates success in the prevention of adverse events and decline in re-hospitalisation rates, while a $<30\%$ decrease in NT-proBNP suggests a worse prognosis.¹⁵

Although mechanisms responsible for the progression of heart failure are not fully known, programmed cell death of cardiomyocytes (i.e. apoptosis, necroptosis) plays a role

in the pathogenesis of heart failure.¹⁰ For instance, in rat experimental models, it was shown that prevention of death of cardiac myocytes mostly precluded cardiac dilatation, and contractile dysfunction.¹¹ In another study, prevention of apoptosis with pharmacologic interventions is another evidence indicating the role of cardiomyocyte apoptosis in the pathogenesis of heart failure.^{12,13} All of these studies especially demonstrated the impact of apoptosis in end-stage heart failure. However; adequate number of studies and data displaying the correlation between acute episodes of decompensated heart failure and apoptosis are lacking.

When compared with admission values, a statistically insignificant increase was detected on the 7th day apoptotic levels of our patients ($p<0.126$). Genetic and pharmacologic studies performed on animal models substantiate the crucial role of apoptosis on the development and progression of heart failure. Wencker et al. demonstrated that very low levels of chronic cardiomyocyte apoptosis could induce fatal dilated cardiomyopathy in hearts of transgenic mice which conditionally expressed active caspase.¹⁶ In cases where cell death is inhibited by a caspase inhibitor, LV dilatation and functional loss can be prevented.^{13,14,16}

Niessner et al. compared BNP and apoptosis markers in patients with advanced heart failure.¹⁴ Mortality rate was 26% during the median follow-up time of 16 months. In this study, it was concluded that mortality in the long run can be predicted at discharge by apoptosis levels rather than by BNP values. In a study in-patient mortality rates were found to be increased in patients whose NT-proBNP levels were $>6.000\text{pg/ml}$ ($p<0.001$). However, any significant correlation between in-patient mortality and admission apoptosis values could not be detected.

Also, when we analysed the association with in-patient mortality and NT-proBNP and apoptosis values, only in deceased patients the levels of 7th day apoptosis markers rose significantly relative to admission values ($p<0.002$), while any significant change between admission and the 7th day NT-proBNP values was not observed ($p<0.906$). The results remind us of the need to design apoptotic activity studies similar to those performed by Niessner et al.¹⁴ related to the prediction of mortality rates in the long run. In our study group with deceased patients, a statistically significant but still relatively lower percent increases in the levels of apoptosis markers were observed ($p<0.002$).

We also observed a statistically significant decline in NT-proBNP values of our patients ($p<0.012$), in contrast to statistically insignificant increases in the levels of

apoptotic markers ($p < 0.12$). Difference between levels of NT-proBNP and apoptosis values can be explained by rapid biochemical circulation of NT-proBNP in line with clinical improvement, compared to more persistent, relatively slower, subtle and prolonged apoptotic processes with different pathogenic mechanisms.^{6,14,17}

NT-proBNP levels were found to be strong predictive factors for heart failure, although intergroup differences were observed. In our study, admission NT-proBNP values predicted in-patient mortality, which is contrary to apoptosis values. In-patient NT-proBNP values of $>6.000\text{pg/ml}$ are associated with in-patient mortality rates ($p < 0.001$). Apoptosis levels detected on admission are not apparently the determining factor for mortality. However, because of a tendency for increased levels of apoptotic markers detected during in-patient monitoring ($p < 0.12$) and also their significant levels in dying patients, these markers have been associated with mortality ($p < 0.002$). The impact of apoptosis on mortality rates in the mid and long-term should be investigated and supported in further studies.

In our study a statistically significant increase was noted in apoptosis, especially in patients on dopamine therapy, on admission ($p < 0.035$). Accelerated apoptotic processes induced by dopamine might be attributed to the presence of more advanced stage of heart failure in these patients with worse clinical and haemodynamic symptoms, delayed recovery of apoptotic processes or adverse effects of dopamine per se. Also, dopamine treatment leads to an increase in apoptotic activity since this is stimulated by orthosympathic activation. In fact, a statistically significant negative correlation was found between left LVEF measured echocardiographically and on the seventh day-apoptosis values ($r = -0.414$; $p < 0.003$). This negative correlation might be associated with decreased cardiomyocyte reserve. The correlation between 7thday-apoptosis levels and LV diastolic diameter was also statistically significant ($r = 0.481$; $p < 0.001$). A statistically weak correlation existed between admission NT-proBNP values and CRP ($r = 0.273$; $p < 0.048$). This is probably related to bad remodelling and increased inflammatory activity. But all anti-apoptotic treatment modalities such as a Ca-sensitiser inotropic agent levosimendan, caspase and/or Poly Adp Ribose Polymerase (PARP) inhibition, stimulation of also activated clotting time (ACT) known as "protein kinase B" (PKB/ACT) pathway, inhibition of Bax using antisense technology, and Adenine Nucleotide Transporter (ANT) antibodies can individually exert beneficial therapeutic effects in these situations. Some studies have demonstrated anti-apoptotic, and bad remodelling

corrective effects of PARP inhibitors.^{16,18-22}

In spironolactone users, a decline in apoptotic activity approaching a statistically significant level has been observed ($p = 0.07$). An animal experiment demonstrated that spironolactone treatment had decreased apoptotic activity in neonatal mice hearts.¹³

It was established that apoptotic process progresses relatively slowly in beta-blocker users compared to non-users of beta-blockers ($p < 0.02$). Favourable effects of beta-blocker and spironolactone usage on apoptotic process have been observed ($p < 0.02$ and $p < 0.07$, respectively). Contrary to our expectations, angiotensin converting enzymes (ACEs), angiotensin II receptor blockers (ARBs), and statins did not exert favourable effects on this process. Also, we did not determine a significant decline in NT-proBNP values of the patients with these drugs.

A study indicated that carvedilol prevented ischaemia/reperfusion induced cardiomyocyte apoptosis in chronic heart failure.²³

As a potential mechanism, the same study stated that in heart failure, the expression of norepinephrine, which is induced by the growth of terminally differentiated cells, and also oxidative stress might stimulate the apoptotic process whose effects could be mediated by $\alpha 1$, β , and $\beta 2$ receptors.²³⁻²⁵

In our study, apoptosis levels did not change in beta-blocker users, while its levels rose statistically significantly in the non-users. In subgroup analyses, observed higher apoptosis levels were in diabetics and male patients. We think that large-scale investigations should be performed on this issue.

The limitations of the study are its monocentric nature, modest sample size, and descriptive, observational non-randomised design. Besides, we did not collect follow-up data to evaluate mid-term and long-term outcomes. Lastly, the study subgroups are minor in size.

Conclusion

NT-proBNP on admission may be an important risk predictor in decompensated heart failure exacerbations during hospitalisation. In-patient NT-proBNP values of $>6.000\text{pg/ml}$ on admission and apoptotic activity levels on the 7th day were associated with in-patient deaths. Beta-blocker therapy positively affected the process of apoptosis inhibition. Dopamine infusion in the treatment group during hospitalisation significantly increased apoptotic activity, whereas there was a trend towards decreased apoptotic activity levels with spironolactone.

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