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# Predictive Value of Circulating Apoptotic Microparticles in Patients with Ischemic Symptomatic Moderate-To-Severe Chronic Heart Failure

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## Abstract

**Aim:** To evaluate the prognostic value of circulating CD31+/annexin V+ Microparticles (MPs) for cumulative survival in patients with ischemic Chronic Heart Failure (CHF).

**Methods:** A total of 154 patients with ischemic symptomatic moderate-to-severe CHF were enrolled in the study on discharge from the hospital. Observation period was up to 3 years. Blood samples for biomarkers measurements were collected. Flow cytometry analysis for quantifying the number of CD31+/annexin V+ MPs was used. CD31+/annexin V+ MPs number for cumulative survival cases due to CHF were tested. Additionally, all-cause mortality, and CHF-related death were examined.

**Results:** During a median follow-up of 2.18 years, 21 participants died and 106 subjects were hospitalized repetitively. Medians of circulating levels of CD31+/annexin V+ MPs in patients who survived and subjects who died were 0.286 n/mL (95% confidence interval [CI] = 0.271-0.309 n/mL) and 0.673 n/mL (95% CI = 0.65-0.74 n/mL) (P<0.001). Number of circulating MPs was distributed into Quartiles (Q): Q1 (< 0.341 n/mL), Q2 (0.342-0.514 n/mL), Q3 (0.521-0.848 n/mL), and Q4 (> 0.850 n/mL). ROC analysis has been shown that cut off point of CD31+/annexin V+ MPs number for cumulative survival function was 0.514 n/mL. Area under cure was 0.913 (Std. error = 0.025; 95% CI = 0.863-0.962), sensitivity and specificity were 89.6% and 69.7% respectively. It has been found a significantly divergence of Kaplan-Meier survival curves in patients with high quartile (MPs number >0.514 n/mL) of MPs numbers when compared with low quartiles. Using a stepwise model selection method for multivariable prediction model we investigated that CD31+/annexin V+ MPs number alone and combination of CD31+/annexin V+ MPs number with NT-pro-brain natriuretic peptide (NT-pro-BNP) remained statistically significant predictors for all-cause mortality, CHF-related death, and CHF-related re-hospitalisations, whereas combination of CD31+/annexin V+ MPs with both NT-pro-BNP and left ventricular ejection fraction did not.

Conclusion: Increased circulating CD31+/annexin V+ MPs associates with increased 3-year CHF-related death, all-cause mortality, and risk for recurrent hospitalization due to CHF.

**Keywords:** Apoptotic microparticles; Chronic heart failure; Survival; Hospitalization; Prognosis

## Introduction

Chronic heart failure (CHF) is considered as a leading cause of morbidity and mortality in worldwide [1]. Endothelial dysfunction has been shown to play a critical role in the clinical manifestations of CHF [2]. Recent studies suggested that injure of endothelial monolayer due to any reasons leads to dramatic increase of circulating level of endothelial-derived apoptotic Microparticles (MPs) [3]. MPs are a heterogeneous population of sub-micronic vesicles that are released in response to cell activationor apoptosis [4]. It has been investigated that MPs represent an intercellular communication and delivery mechanism for the efficient and effective transfer of biological information, which selectively packaged as intracellular material included bioactive lipids, integrins, cytokines, enzymes, mRNA and micro-RNA that lead to reprogramming recipient cells; proatherogenic and pro-thrombotic effects; as well as modulating inflammatory response [5,6]. Increase in circulating MPs is detectable in several cardiovascular diseases, such as acute coronary syndrome, atherosclerosis, dyslipidaemia, hypertension, stroke, atrial fibrillation, as well as sepsis, cancer, lupus erythematosus, chronic kidney disease, type two diabetes mellitus, obesity [7-9]. While MP are sensitivity markers of endothelial dysfunction and tissue remodelling, they are also indicator of an imbalance between pro-angiogenic and anti-angiogenic responses, and they could be used to predict value in cardiovascular disease [3,7,10]. We postulated that CD31+/annexin V+ MP might be discussed as prognostic factors in CHF, but their predictive value in patients with symptomatic ischemic CHF has not been defined. The aimof this study was to evaluate the potential prognostic value of circulating CD31+/annexin V+ MP for cumulative survival in patients with ischemic CHF.

## Methods

### Patient population under study

The study evolved 154 patients (86 males) aged 48 to 62 years with ischemic symptomatic moderate-to-severe CHF. Chronic heart failure was diagnosed according to current clinical guidelines [11]. Table 1 show characteristics of the patients participated in the study. All the

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patients have given their written informed consent for participation in the study. The following are exclusion criteria: Q-wave and non-Q-wave myocardial infarction within 3 months before study entry; severe kidney and liver diseases that may affect clinical outcomes; malignancy; creatinin plasma level above 440 μmol/L; estimated glomerular filtration rate (eGFR) < 35 ml/min/м$^2$; brain injury within 3 months before the enrollment; body mass index above 30 kg/m$^2$ and less15kg/m$^2$; pulmonary edema; tachyarrhythmia; valvular heart disease; thyrotoxicosis; ischemic stroke; intracranial hemorrhage; acute infections; surgery; trauma; all the ischemic events within 3 previous months; inflammations within a previous month; neoplasm; pregnancy; implanted pacemaker, any disorder that may discontinue patient's participation in the study according to investigators; and patient's refusal to participate in the study or to give his consent for it. Observation period was up to 3 years. We analyzed cumulative survival related to CHF, and additionally all-cause mortality was examined.

### Methods for visualization of coronary arteries

Multispiral computed tomography angiography and/or angiographic study have been carried out to verify the ischemic nature of the disease in patients. Multispiral computed tomography angiography has been carried out for all the patients prior to their inclusion in the study. When atherosclerotic lesions of the coronary arteries were verified, patients were subjected to conventional angiographic examination provided indications for revascularization were available. Coronary Artery Disease (CAD) was considered to be diagnosed upon availability of previous angiographic examinations carried out not later than 6 months ago provided no new cardiovascular events occurred for this period. The coronary artery wall structure was measured by means of contrast spiral computed tomography angiography [12] on Somatom Volum Zoom scanner (Siemens, Erlangen, Germany) with two detector rows when holding patients breathe at the end of breathing in. After preliminary native scanning, non-ionic contrast Omnipak (Amersham Health, Ireland) was administered for the optimal image of the coronary arteries. To reconstruct the image, 0.6-mm-width axial tomographic slices were used.

### Assessment of hemodynamics

Transthoracic ultrasonic echocardiography was performed according to a conventional procedure on ACUSONapparatus, SIEMENS, Germany, in B-mode regimen and tissue Doppler echocardiography regimen from parasternal, subcostal, and apical positions over the short and long axis with sensor P of 5 MHz. Left ventricular end-diastolic and end-systolic volumes were measured by modified Simpson's planimetric method; and they were measured by cylinder method if severe failure of local myocardial contractility was verified. Left Ventricular Ejection Fraction (LVEF) was assessed in compliance with the requirements of American Society of Echocardiography [13]. Tissue Doppler echocardiography was carried out in 4-, 3- and 2-chamber projections in each of 16 segments of the left ventricle and in 4 spots of the mitral annulus: at the base of posterior septal, lateral, inferior, and anterior left ventricular walls [14]. Peak systolic (Sm), early diastolic (Em), and late diastolic (Am) myocardial velocities were measured in the mitral annulus area, followed by calculating velocity of early diastolic left ventricular filling (E) to Am (E/Am) ratio and to Em (E/Em) ratio.

### Calculation of glomerular filtration rate

Calculation of glomerular filtration rate (GFR) was carried out using MDRD-6 formula [15].

### Blood sampling, measurement of NT-pro-BNP, total cholesterol and its fractions

Blood sampling for further biochemical and biomarkers measurements was obtained obligatory before visualization procedures in the morning (at 7-8 a.m.) into cooled silicone test tubes. Samples were processed according to the recommendations of the manufacturer of the analytical technique used. They were centrifuged upon permanent cooling at 6,000 rpm for 30 minutes. Then, plasma was refrigerated immediately to be stored at a temperature not higher than -35˚C. Circulating N-terminal pro-brain natriuretic peptide (NT-pro-BNP) level was measured by immune electro chemoluminescent assay using sets by R&D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). Concentrations of Total Cholesterol (TC) and cholesterol of High-Density Lipoproteins (HDLP) were measured by fermentation method. Concentration of cholesterol of Low-Density Lipoproteins (LDL-C) was calculated according to the Friedewald formula (1972).

### Assay of circulating CD31+/Annexin V+ endothelial-derived apoptotic microparticles

Circulating MPs were isolated from 5 ml of venous citrated blood drawn from the fistula-free arm. Platelet-Free Plasma (PFP) was separated from whole blood and then was centrifugated at 20,500 × rpm for 30 min. MPs pellets were washed with DMEM (supplemented with 10 μg/ml polymyxin B, 100 UI of streptomycin, and 100 U/ml penicillin) and centrifuged again (20,500 rpm for 30 min). The obtained supernatant was extracted, and pellets were resuspended into the remaining 200 μl of supernatant. PFP, MP, pellet, and supernatant were diluted five-, 10-, and five-fold in PBS, respectively. Apoptotic MPs were phenotyped by flow cytofluorimetry by phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (BD Biosciences, USA) followed by incubation with Fluorescein Isothiocyanate (FITC)-conjugated annexin V (BD Biosciences, USA) per HD-FACS (High-Definition Fluorescence Activated Cell Sorter) methodology. The samples were incubated in the dark for 15 min at room temperature according to the manufacturer's instructions. The samples were then analyzed on a FC500 flow cytometer (Beckman Coulter) after 400 μL annexin-V binding buffer was added. For each sample, 500 thousand events have been analyzed. CD31+/annexin V+ MP gate was defined by size, using 0.8 and 1.1 mm beads (Sigma, St Louis, MO, USA). Apoptotic microparticles were defined as CD31+/annexin V+ MPs positively labeled for CD31 and annexin V (CD31+/annexin V+) [16].

### Statistical analysis

Statistical analysis of the results obtained was carried out in SPSS system for Windows, Version 22 (SPSS Inc, Chicago, IL, USA). The data were presented as Mean (M) and error of mean (± m) or 95% Confidence Interval (CI); Median (Me) and interquartile range. To compare the main parameters of patients' groups (subject to the type of distribution of the parameters analyzed), one-tailed Student t-test or Shapiro-Wilk U-test were used. To compare categorical variables between groups, Chi2 test ($\chi^2$) and Fisher F exact test were used. The circulating CD31+/annexin V+ MP and NT-pro-BNP level in the blood failed to have a normal distribution, while distribution of the total cholesterol and cholesterol fractions had a normal character (estimated by means of Kolmogorov-Smirnovtest) and was not subjected to any mathematical transformation. The factors, which could be associated potentially with circulating CD31+/annexin V+ MPs, were determined by logistic regression analysis. Receive Operation Curve (ROC) analysis was performed to identify the optimal cutoff points of the

CD31+/annexin V+ MPsnumber with predicted value. Odds Ratio (OR) and 95% confidence interval (95% CI) were calculated for all the independent predictorsof survival of the patients. A calculated difference of P<0.05 was considered significant.

## Results

### General characteristics of study patient population

During a median follow-up of 2.18 years, 21 participants died and CHF-related death was defined in 18 patients. Additionally, 106 subjects were hospitalized repetitively due to advance CHF (17 cases in died cohort and 89 cases in survival cohort). Table 1 shows a general characteristic of the patients included in the study. As one can see from Table 1, no substantial age and gender differences were found among persons who died and survived, as well as differences in Body Mass Index (BMI), Glomerular Filtration Rate (GFR), glycated hemoglobin (HbA1c), fasting blood glucose level, blood creatinine level, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and High-Density Lipoprotein Cholesterol (HDL-C), numerous of coronary vessels damaged. No difference was found between the two cohorts in systemic office Blood Pressure (BP) and Heart Rate (HR). Documented incidence of Type 2 Diabetes Mellitus (T2DM) in patients of the two cohorts was 38.1% and 33.8% (P = 0.06). Note that there was not a statistically significant change in peak velocity of early diastolic left ventricular filling to late diastolic myocardial velocity ratio (E/Am) and peak velocity of early diastolic left ventricular filling to early diastolic myocardial velocity ratio (E/Em) between the two cohorts, while decrease in the left ventricular ejection fraction value was quite

| Variables | Subjects who died (n=21) | Subjectswho survived (n=133) | P value |
|---|---|---|---|
| Age, years | 57.20±6.70 | 59.50±7.30 | 0.86 |
| Males, n (%) | 12 (57.1%) | 67 (50.3%) | 0.44 |
| Arterial hypertension, n (%) | 12 (57.1%) | 61 (45.9%) | 0.48 |
| Hyperlipidemia, n (%) | 9 (42.8%) | 52 (39.1%) | 0.66 |
| T2DM, n (%) | 8 (38.1%) | 45 (33.8%) | 0.82 |
| Adherence to smoking, n (%) | 7 (33.3%) | 24 (29.3%) | 0.81 |
| NYHA Class | | | |
| II Class | 6 (28.6%) | 35 (26.3%) | 0.78 |
| III Class | 9 (42.8%) | 65 (48.9%) | 0.76 |
| IV Class | 6 (28.6%) | 33 (24.8%) | 0.87 |
| BMI, kg/m2 | 23.7 (95% CI=22.5–27.3) | 24.2 (95% CI=22.0–27.9) | 0.82 |
| GFR, mL/min/1.73 m2 | 82.1 (95% CI=69.9–93.1) | 85.2 (95% CI=70.3–112.5) | 0.74 |
| HbA1c, % | 6.3 (95% CI=4.4-9.0) | 7.0 (95% CI=4.3-9.2) | 0.42 |
| Fasting blood glucose, mmol/L | 4.80 (95% CI=3.6-8.5) | 5.40 (95% CI=3.4-9.1) | 0.29 |
| Creatinine, μmol/L | 70.5 (95% CI=59.6–88.3) | 74.9 (95% CI=65.1–90.3) | 0.26 |
| Totalcholesterol, mmol/L | 5.3 (95% CI=4.6-6.0) | 5.0 (95% CI=4.2-5.8) | 0.34 |
| LDL-C, mmol/L | 3.60 (95% CI =3.20–4.18) | 3.02 (95% CI=2.80–3.90) | 0.46 |
| HDL-C, mmol/L | 0.94 (95% CI = 0.92–1.06) | 0.88 (95% CI = 0.82–0.97) | 0.49 |
| NT-pro-BNP, pg /mL | 1533.6 (95% CI644.5 – 2560.6) | 1031.2 (95% CI 704.8 – 1560.7) | 0.038 |
| Systolic BP, mm Hg | 129±4 | 135±5 | 0.52 |
| Heart rate, beats per 1 min. | 76±6 | 68±3 | 0.64 |
| LVEF, % | 42.80±0.76 | 55.40±0,80 | 0.044 |
| E/Am, U | 16.6±0.94 | 16.5±1.20 | 0.88 |
| E/Em, U | 16.6±1.00 | 16.6±0.84 | 0.82 |
| Number of coronary arteries affected | | | |
| One-vessel lesion, n (%) | 5 (23.8%) | 24 (18.0%) | 0.12 |
| Two-vessel lesion, n (%) | 8 (38.1%) | 54 (40.1%) | 0.15 |
| Three- and multi-vessel lesion, n (%) | 8 (38.1%) | 55 (41.4%) | 0.11 |
| ACEI / ARAs, n (%) | 21 (100%) | 133 (100%) | - |
| Acetylsalicylic acid, n (%) | 19 (90.5%) | 121 (91.0%) | 0.86 |
| Other antiaggregants, n (%) | 2 (9.5%) | 12 (9.0%) | 0.72 |
| Statins, n (%) | 14 (66.7%) | 80 (60.2%) | 0.68 |
| metformin, n (%) | 8 (38.1%) | 45 (33.8%) | 0.77 |
| diuretics, n (%) | 18 (85.7%) | 121 (91.0%) | 0.66 |
| Mineralcorticoid receptors antagonists, n (%) | 9 (42.9%) | 70 (52.6%) | 0.52 |

Note: CI: Confidence Interval; CAD: Coronary Artery Disease, T2DM: Type Two Diabetes Mellitus,GFR: Glomerular Filtration Rate, HDL-C: High-Density Lipoprotein Cholesterol, LDL-C: Low-Density Lipoprotein Cholesterol, BP: Blood Pressure, BMI: Body Mass Index, NYHA: New York Heart Association, BNP: Brain Natriuretic Peptide: LVEF: Left Ventricular Ejection Fraction, U: Unit, Em: Early Diastolic Myocardial Velocity, Am: Late Diastolic Myocardial Velocity: E: Peak Velocity Of Early Diastolic Left Ventricular Filling, ACEI: Angiotensin-Converting Enzyme Inhibitor; Aras: Angiotensin-2 Receptors Antagonists

**Table 1:** General characteristic of patients participating in the study

anticipated in the setting in patients who died. At the same time, the level of circulating NT-pro-BNP was statistically significantly higher in patients who died than in persons who survived. When analyzing details of pharmacotherapy, no substantial differences were found between the two cohorts with regard to administration of the majority of drugs.

## Circulating CD31+/annexin V+ MPs level in study patient population

Medians of circulating levels of CD31+/annexin V+CD31+/annexin V+ MPs in cohorts patients who survived and patients who died were 0.286 n/mL (95% Confidence Interval [CI] = 0.271-0.309 n/mL) and 0.673 n/mL (95% CI = 0.65-0.74 n/mL) (P <0.001). Number of circulating CD31+/annexin V+ MPs was distributed into Quartiles (Q): Q1 (<0.341 n/mL), Q2 (0.342-0.514 n/mL), Q3 (0.521-0.848 n/mL), and Q4 (>0.850 n/mL). The data suggested that CD31+/annexin V+ MPs number in plasma were directly related to New York Heart Association (NYHA) class of CHF (r = 0.514, P = 0.001), NT-pro-BNP (r = 0.416, P = 0.001), T2DM (r = 0.402, P = 0.003), multi-vessel lesion of coronary arteries (r = 0.362, P = 0.001), E/Am (r = 0.360, P = 0.001), E/Em (r = 0.344, P = 0.001), gender (r = 0.318, P < 0.001 for male), total cholesterol (TC) (r = 0.313, P = 0.001), age (r = 0.275, P = 0.001), smoking (r = 0.212, P = 0.001) and inversely to LVEF (r = -0.496, P = 0.001) and eGFR (r = -0.408, P = 0.003). No significant association between the levels of circulating CD31+/annexin V+ MPs with fasting plasma glucose, HbA1c, means systolic and diastolic BP, premature CAD in family anamnesis, and medications for both cohorts of the patients was found.

## The predictive value of CD31+/annexin V+ MP number in study patient population

The optimum cut-off point for CD31+/annexin V+ MP number in circulation is determined by the relative importance of the sensitivity and specificity of the test. Receive Operation Curve (ROC) analysis has been shown that cut-off point of CD31+/annexin V+ MPs number for cumulative survival function was 0.514 n/mL (Figure 1). Area under cure was 0.913 (Std. error = 0.025; 95% CI = 0.863-0.962), sensitivity and specificity were 89.6% and 69.7% respectively. Iterations between sensitivity and specificity of CD31+/annexin V+ MPs cut-off point level for other clinical outcomes in study patient population are presented Table 2. For all occasions the model was robust and it has provided a significant results using optimal cut-off point of CD31+/annexin V+ MPs.

It has been found a significantly divergence of Kaplan-Meier survival curves in patients with high quartile ( > 0.514 n/mL) of CD31+/annexin V+ MPs numbers when compared with low quartiles (Q1-Q3) (Figure 2). The curves divergence of events accumulation reached a statistical significance in 50 weeks of observation period (P <0.001 for all cases). No statistically significance differences between survival in patient cohorts with Q1 and Q2, as well as Q2 and Q3 in numbers of CD31+/annexin V+ MPs were found. The divergence between two cohorts with CD31+/annexin V+ MPs numbers in Q1 and Q3 was reached be able significance in 60 weeks after study entry.

Multivariate logistic regression was used to assess whether any combination of assays was able to better discriminate between survival and died patients. In the logistic regression analysis, the main factors independently related with cumulative mortality and CHF-related re-hospitalisations were MPs, NT-pro-BNP, NYHA class, LVEF, T2DM, and three- and multi-vessel lesion. Circulating MPs number independently predicted all-cause mortality (OR = 1.58; 95% CI = 1.20–1.88; P = 0.001), CHF-related death (OR = 1.22; 95% CI 1.12–1.36; P < 0.001), and also CHF-related rehospitalisation (OR = 1.20; 95% CI = 1.11 – 1.32; P < 0.001) within 3 years of observation period (Table 3). NYHA class, NT-pro-BNP and LVEF remained statistically significant for all categories: all-cause mortality, CHF-related death, and CHF-related re-hospitalisations, whereas T2DM and three- and multi-vessel lesion for all variables did not.

Using a stepwise model selection method for multivariable prediction model we n investigated the summary effect of any combinations of CD31+/annexin V+ MPs, NT-pro-BNP, and LVEFon all-cause mortality, CHF-related death, and CHF-related re-hospitalisations. We found that CD31+/annexin V+ MPs number alone and combination of CD31+/annexin V+ MPs number with NT-pro-BNP remained statistically significant predictors for all-cause mortality (B-coefficient=5.38, p= 0.001, and B-coefficient = 6,32, p = 0.001 respectively), CHF-related death (B-coefficient = 4.34, p= 0.001, and B-coefficient = 5.11, p = 0.001 respectively), and CHF-related re-hospitalisations (B-coefficient = 4.88, p = 0.001, and B-coefficient = 3,26, p = 0.001 respectively), whereas combination of CD31+/annexin V+ MPs with both NT-pro-BNP and LVEF did not (B-coefficient = 0.016, p = 0.72, and B-coefficient = 0,022, p = 0.58, and B-coefficient = -0,021, p = 0.52 respectively (Predicted Models)). A stepwise model selection method demonstrated that NYHA class, LVEF, T2DM and three- and multi-vessel lesion of coronary arteries added to combination of CD31+/annexin V+ MPs and NT-pro-BNP do not offer any additional information to discriminate between survived and died patients with symptomatic ischemic CHF (B-coefficient of 0.14; 0.018; 0.086; and 0.016 respectively; p-values of 0.86; 0.65; 0.58; and 0.56 respectively).

## Discussion

Circulating CD31+/annexin V+ MPs play a pro-inflammatory and pro-coagulant detrimental role in the vascular dysfunction that is a key mechanism in the development and progression of a wide range of cardiovascular diseases [17]. Recent studies revealed that CD31+/annexin V+ MPs may trigger endothelial dysfunction by disrupting production of nitric oxide release from vascular endothelial cells and subsequently modifying vascular tone [3,7]. Circulating CD31+/

| | Cut-off point, n/mL | Sensitivity, % | Specificity, % | AUC (95% CI) | P-value |
|---|---|---|---|---|---|
| CHF-related death | 0.514 | 99.3 | 56.2 | 0.906 (0.843-0.970) | 0.001 |
| CHF-related hospitalization | 0.514 | 87.5 | 65.0 | 0.86 (0.796-0.924) | 0.001 |
| All-cause mortality | 0.514 | 99.6 | 57.4 | 0.906 (0.846-0.965) | 0.001 |

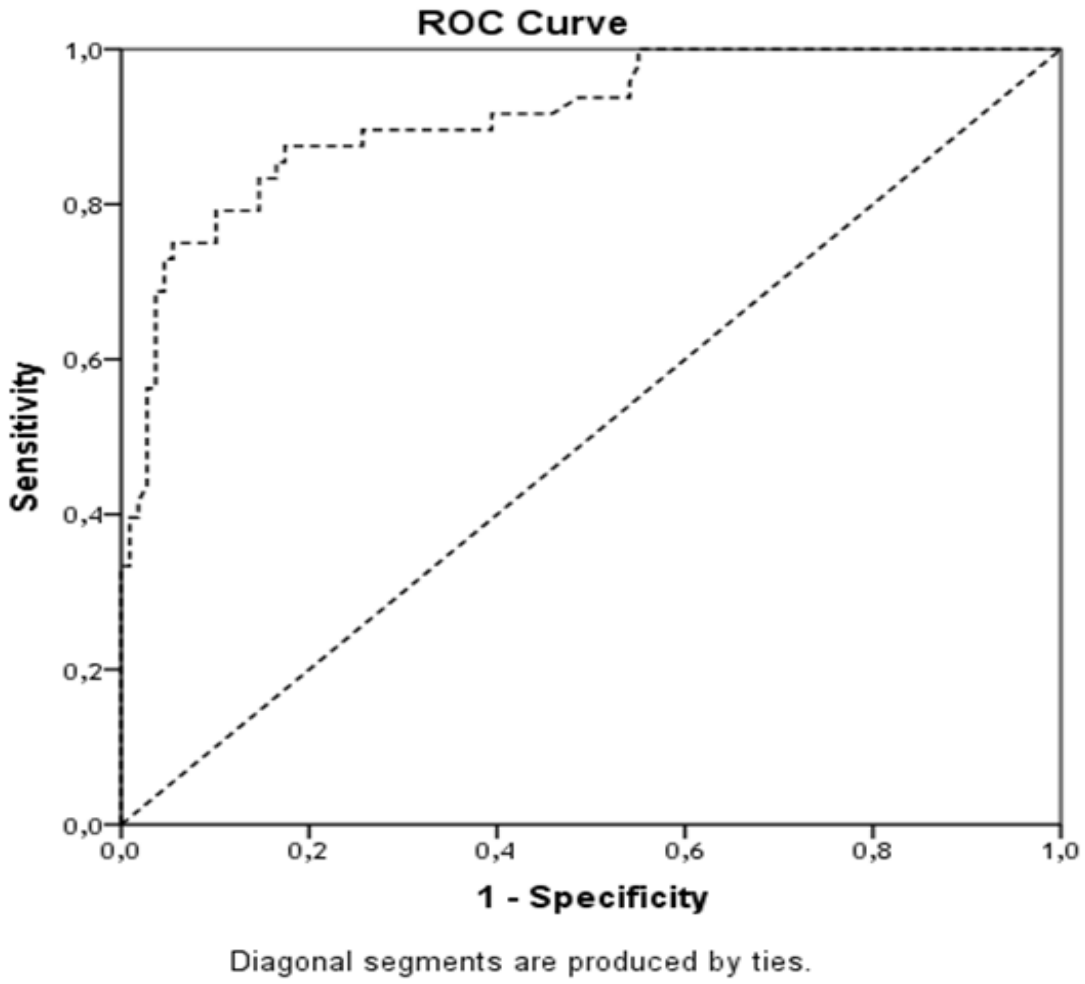
Note: AUC: Area Under Curve; CI: Confidence Interval

**Table 2:** Iterations between sensitivity and specificity of CD31+/annexin V+ MPs cut-off point for clinical outcomes in study patient population. Results of the Receive Operation Curve analysis

| Variables | All-cause mortality | | | CHF-related death | | | CHF-related rehospitalisation | | |
|---|---|---|---|---|---|---|---|---|---|
| | OR | 95% CI | P | OR | 95% CI | P | OR | 95% CI | P |
| CD31+/annexin V+ MPs | 1.58 | 1.20–1.88 | 0.001 | 1.22 | 1.12–1.36 | 0.001 | 1.20 | 1.11 – 1.32 | 0.001 |
| NYHA class | 1.12 | 1.01–1.24 | 0.05 | 1.18 | 1.05–1.30 | 0.001 | 1.12 | 1.07 – 1.22 | 0.001 |
| NT-pro-BNP | 1.09 | 1.02–1.16 | 0.002 | 1.42 | 1.22–1.73 | 0.006 | 1.44 | 1.28–1.67 | 0.002 |
| LVEF | 1.06 | 1.01–1.12 | 0.001 | 1.15 | 1.12–1.18 | 0.014 | 1.22 | 1.07–1.45 | 0.016 |
| T2DM | 1.05 | 1.01–1.11 | 0.001 | 1.03 | 0.93–1.10 | 0.32 | 1.04 | 0.97–1.06 | 0.42 |
| three- and multi-vessel lesion | 1.02 | 0.88–1.09 | 0.56 | 1.01 | 0.92–1.07 | 0.27 | 1.14 | 1.03–1.26 | 0.012 |

Note: OR: Odds Ratio; CI: Confidence Interval; LVEF: Left Ventricular Ejection Fraction; BNP: Brain Natriuretic Peptide; T2DM: Type Two Diabetes Mellitus

**Table 3:** Variables independently related to 3-years all-cause mortality, CHF-related death, and CHF-related rehospitalisation, obtained by Logistic Regression Analysis.




| Area Under the Curve | | | | |
|---|---|---|---|---|
| Test Result Variable(s): Endothelial-derived micropaticles CD31+annexin V+ | | | | |
| Area | Std. Error | Asymptotic Sig. | Asymptotic 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound |
| 0.913 | 0.025 | 0.001 | 0.863 | 0.962 |
| The test result variable(s): CD31+annexin V+ apoptotic micropaticles has at least one tie between the positive actual state group and the negative actual state group. | | | | |

**Figure 1:** Reliability of the model included CD31+/annexin V+ MPs number for cumulative survival in study patient population. Results of the Receive Operation Curve (ROC) analysis

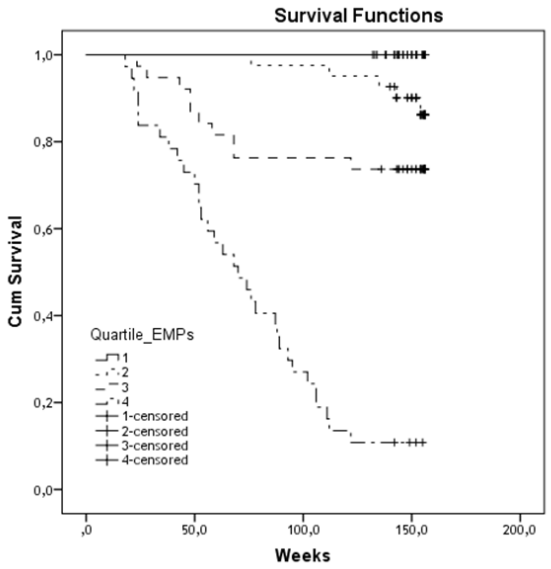



**Figure 2:** Results of Kaplan-Meier survival analysis: The cumulative survival in four patient cohorts with different quartiles of CD31+/annexin V+ MPs numbers in isolates

annexin V+ MPs affect both pro-inflammatory and pro-atherosclerotic processes, promote coagulation and inflammation, and also modulate angiogenesis and apoptosis in endothelial cells [18-20]. Because endothelium is one of the primary targets of circulating micro vesicles, CD31+/annexin V+ MPs have been considered as biomarkers of vascular injury, inflammation and stage of progression of cardiovascular diseases. Recent study has revealed an association between circulating apoptotic MPs labelled as CD31+/annexin V+cells with cardiovascular outcomes [10]. However, no previous study has mentioned the possible predicted role of circulating CD31+/annexin V+ MPs levels in the CHF. In our investigation we found a significantly increase of CD31+/annexin V+ MPs level in circulation in ischemic CHF patients who died when compared with those who survived. Quartile distribution of CD31+/annexin V+ MPs with further cumulative survival analysis with Kaplan-Meier has been shown a significant divergence between curves in Q4 CD31+/annexin V+ MPs and other quartiles. Therefore, cut-off point for survived and died patients with different plasma level of CD31+/annexin V+ MPs was 0.514 n/mL, and it was equal number cells that divided Q4 and Q3 in CD31+/annexin V+ MPs. Using this data we found that increased CD31+/annexin V+ MPs number more 0.514 n/mL independently predicted all-cause mortality, CHF-related death, and also CHF-related rehospitalisation ($P<0.001$ for all cases) within observation period. Multivariable prediction model has been shown a high decremented potential of CD31+/annexin V+ MPs alone and in combination with NT-pro-BNP in CHF patients during 3 years after baseline. These findings suggest that increased CD31+/annexin V+ MPs number might improve the predictive value of contemporary model in CHF based on clinical performances and NT-pro-BNP measurements. Although the cellular mechanism of action of CD31+/annexin V+ MPs largely remains unclear, we believe that increased CD31+/annexin V+ MPs in CHF may reflect a reduced vascular repair capacity and severity of endothelial dysfunction that is, probably, considered as staging disease. In this study, levels of CD31+/annexin V+ MsP and NT-pro-BNP were sufficient to predict long-term changes significant as independent factors in cumulative survival, re-hospitalisation due to CHF, and CHF-related death. It should emphases that while CD31+/annexin V+ MPs have large diagnostic potential as biomarkers in cardiovascular diseases and cancer; however, due to current technological limitations in purification of CD31+/annexin V+ MPs and an absence of standardized methods of detection, the role of CD31+/annexin V+ MPs became controversial [21-23].There are data elucidated that a large pool of nanoparticles is produced after blood sampling due to fragmentation of blood cells [24]. Indeed, such a possibility is not excluded, that in our opinion should be taken into account when interpreting the data. New studies with more statistical powerful are required. Knowledge of the functional properties of CD31+/annexin V+ MPs will contribute to a better understanding of the pathological mechanisms of communication between cells and CHF progression, because CD31+/annexin V+ MPs may be an attractive prognostic biomarker for CHF.

## Conclusion

Among patients with symptoms of CHF, increased circulating CD31+/annexin V+ MPs number associates with increased 3-year CHF-related death, all-cause mortality, and risk for recurrent hospitalization due to CHF.

## Ethical Principles

The study was approved by the local ethics committee of State Medical University, Zaporozhye, Ukraine. The study was carried out in conformity with the Declaration of Helsinki.

## Study Restrictions

This study has some restrictions. The authors believe that a greater cohort of patients with more incidences detected is desirable to improve the power of the study. It is necessary to note that large pool of nanoparticles might be produced after blood sampling. We believe that these risks are systemic, and to minimize them, we refused to freeze the blood samples before measurement of microparticles. The authors suppose that these restrictions might have no significant impact on the study data interpretation.

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