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Predictive value of circulating apoptotic microparticles in patients with ischemic symptomatic moderate-to-severe chronic heart failure

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 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/mL (95% confidence interval [CI]=0.271–0.309/mL) and 0.673/mL (95% CI=0.65–0.74/mL) ($P<0.001$). Number of circulating MPs was distributed into Quartiles (Q): Q1 (<0.341/mL), Q2 (0.342–0.514/mL), Q3 (0.521–0.848/mL), and Q4 (>0.850/mL). Receiver Operation Curve (ROC) analysis has been shown that cut off point of CD31⁺/annexin V⁺ MPs number for cumulative survival function was 0.514/mL. Area under curve was 0.913 (Standard 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/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.

Key words: apoptotic microparticles, chronic heart failure, prognosis.

Background

Chronic heart failure (CHF) is considered as a leading cause of morbidity and mortality in worldwide (Roger V.L., 2010). Endothelial dysfunction has been shown to play a critical role in the clinical manifestations of CHF (Matsuzawa Y. et al., 2013). Recent studies suggested that injury of endothelial monolayer due to any reasons leads to dramatic increase of circulating level of endothelial-derived apoptotic microparticles (MPs) (Horstman L.L. et al., 2004). MPs are a heterogeneous population of submicronic vesicles that are released in response to cell activation or apoptosis (Hristov M. et al., 2004). 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, matrix ribonucleic acid (mRNA) and micro-RNA that lead to reprogramming recipient cells; proatherogenic and prothrombotic effects; as well as modulating inflammatory response (Mallat Z. et al., 2000; Norling L.V., Dalli J., 2013). Increase in circulating MPs is detectable in several cardiovascular diseases, such as acute coronary syndrome, atherosclerosis, dyslipidae-

mia, hypertension, stroke, atrial fibrillation, as well as sepsis, cancer, lupus erythematosus, chronic kidney disease, type two diabetes mellitus, obesity (Pirro M. et al., 2006; Huang P.H. et al., 2010; Jesel L. et al., 2013). While MPs 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 (Sinning J.M. et al., 2011). It has been postulated that CD31⁺/annexin V⁺ MPs might be discussed as prognostic factors in CHF, but their predictive value in patients with symptomatic ischemic CHF has not been defined. The aim of this study was to evaluate the potential prognostic value of circulating CD31⁺/annexin V⁺ MP for cumulative survival in patients with ischemic CHF.

Methods

The study evolved 154 patients (86 males) aged 48 to 62 years with ischemic symptomatic moderate-to-severe CHF. CHF was diagnosed according to current clinical guidelines (McMurray J.J.V. et al., 2012). **Table 1** shows characteristics of the patients participated in the study. All the patients have given their written informed consent for participa-

tion 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; creatinine plasma level above 440 $\mu\text{mol/L}$; estimated glomerular filtration rate (GFR) <35 ml/min/m²; brain injury within 3 months before the enrollment; body mass index above 30 kg/m² and less 15 kg/m²; 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.

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

Table 1 General characteristic of patients participating in the study

Variables	Subjects who died (n=21)	Subjects who survived (n=133)	p
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, n (%)	6 (28.6)	35 (26.3)	0.78
III Class, n (%)	9 (42.8)	65 (48.9)	0.76
IV Class, n (%)	6 (28.6)	33 (24.8)	0.87
Body mass index, kg/m ²	23.7 (95% CI=22.5–27.3)	24.2 (95% CI=22.0–27.9)	0.82
GFR, mL/min/1.73 m ²	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
Total cholesterol, 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% CI 644.5–2560.6)	1031.2 (95% CI 704.8–1560.7)	0.038
Systolic blood pressure, 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
Mineralocorticoid receptors antagonists, n (%)	9 (42.9)	70 (52.6)	0.52

Notes (alphabetically): ACEI – angiotensin-converting enzyme inhibitor; Am – late diastolic myocardial velocity; ARAs – angiotensin-2 receptors antagonists; BNP – brain natriuretic peptide; CAD – coronary artery disease; CI – confidence interval; E – peak velocity of early diastolic left ventricular filling; Em – early diastolic myocardial velocity; GFR – glomerular filtration rate; HbA1c – glycated hemoglobin; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; LVEF – left ventricular ejection fraction; NYHA – New York Heart Association; T2DM – type two diabetes mellitus; U – unit.

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 (Bluemke D.A. et al., 2008) on Somatom Volum Zoom scanner (Siemens, Erlangen, Germany) with two detector rows when holding patients breathe at the end of breathing. 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.

Transthoracic ultrasonic echocardiography was performed according to a conventional procedure on ultrasound scanner (ACUSON, Siemens, Germany) in B-mode regimen and tissue Doppler echocardiography regimen from parasternal, subcostal, and apical positions over the short and long axis with P-transducer of 5 MHz. Left ventricular end-diastolic and end-systolic volumes were measured by modified Simpson's method. Left ventricular ejection fraction (LVEF) was

assessed in compliance with the requirements of American Society of Echocardiography (Schiller N.B. et al., 1989). 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 (Pellerin D. et al., 2003). 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 (GFR) was carried out using MDRD-6 formula (Levey A.S. et al., 2009).

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 min. 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 immunoelectrochemoluminescent assay using sets

by R&D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). Concentrations of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were measured by fermentation method. Concentration of low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula (1972).

Circulating MPs were isolated from 5 ml of venous citrated blood drawn from the fistula-free arm. Platelet-free plasma was separated from whole blood and then was centrifugated at 20,500 rpm for 30 min. MPs pellets were washed with Dulbecco's modified Eagle's medium (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. Platelet-free plasma, MPs, pellet, and supernatant were diluted five-, 10-, and five-fold in PBS, respectively. Apoptotic MPs were phenotyped by flow cytometry 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 MPs were defined as CD31⁺/annexin V⁺ MPs positively labeled for CD31 and annexin V (CD31⁺/annexin V⁺) (Lacroix R. et al., 2013).

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% 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 (χ²) 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 TC and cholesterol fractions had a normal character (estimated by means of Kolmogorov – Smirnov test) 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. Receiver Operation Curve (ROC) analysis was performed to identify the optimal cutoff points of the CD31⁺/annexin V⁺ MPs number with predicted value. Odds ratio (OR) and 95% (CI) were calculated for all the independent predictors of survival of the

patients. A calculated difference of $P < 0.05$ was considered significant.

Results

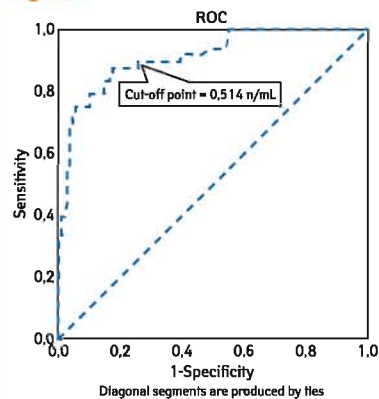
During a median follow-up of 2.18 years, 21 participants died and CHF-related death was defined in 18 patients. Additionally, 106 subjects were hospitalized repetitively due to advance CHF (17 cases in died cohort and 89 cases in survival cohort). **Table 1** shows a general characteristic of the patients 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, GFR, glycated hemoglobin (HbA1c), fasting blood glucose level, blood creatinine level, TC, LDL-C and HDL-C, numerous of coronary vessels damaged. No difference was found between the two cohorts in systemic office blood pressure and heart rate. 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 LVEF value was quite 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.

Medians of circulating levels of CD31⁺/annexin V⁺ MPs in cohorts patients who survived and patients who died were 0.286/mL (95% CI=0.271–0.309/mL) and 0.673/mL (95% CI=0.65–0.74/mL) ($P < 0.001$). Number of circulating CD31⁺/annexin V⁺ MPs was distributed into Quartiles (Q): Q1 (<0.341/mL), Q2 (0.342–0.514/mL), Q3 (0.521–0.848/mL), and Q4 (>0.850/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), 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 estimated GFR ($r = -0.408$, $P = 0.003$). No significant association between the levels of circulating CD31⁺/annexin V⁺ MPs with fasting plasma glucose, HbA1c, means systolic and diastolic blood pressure, premature CAD in family anamnesis, and medications for both cohorts of the patients was found.

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. ROC analysis has been shown that cut-off point of CD31⁺/annexin V⁺ MPs number for cumulative sur-

vival function was 0.514/mL (**Fig. 1**). Area under curve (AUC) was 0.913 (Standard 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.

Figure 1



AUC				
Test Result Variable(s)	Standard error	Asymptotic Significance	Asymptotic 95% CI	
Endothelial-derived MPs CD31 ⁺ annexin V ⁺	0.025	0.001	Lower Bound	Upper Bound
			0.863	0.962

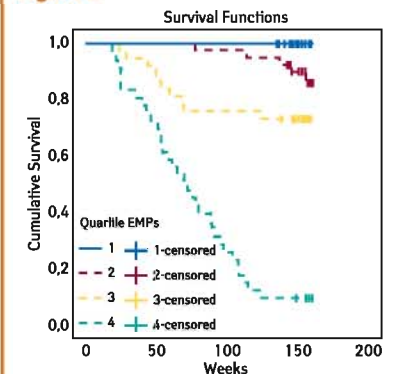
The test result variable(s): CD31⁺/annexin V⁺ apoptotic MPs has at least one tie between the positive actual state group and the negative actual state group.

Reliability of the model included CD31⁺/annexin V⁺ MPs number for cumulative survival in study patient population. Results of the ROC analysis

It has been found a significantly divergence of Kaplan — Meier survival curves in patients with high quartile (>0.514/mL) of CD31⁺/annexin V⁺ MPs numbers when compared with low quartiles (Q1–Q3) (**Fig. 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.

Figure 2



Results of Kaplan — Meier survival analysis: the cumulative survival in four patient cohorts with different Q of CD31⁺/annexin V⁺ MPs numbers in isolates

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 re-hospitalisation (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 investigated the summary effect of any combinations of CD31⁺/annexin V⁺ MPs, NT-pro-BNP, LVEF on all-cause mortality, CHF-related death, and CHF-related re-hospitalisations. We found that CD31⁺/annexin V⁺ MPs number alone (Model 1) and combination of CD31⁺/annexin V⁺ MPs number with NT-pro-BNP

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 ROC analysis

	Cut-off point, n/mL	Sensitivity, %	Specificity, %	AUC (95% CI)	P
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

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

(Model 2) 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 (Model 3) 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). 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 procoagulant detrimental role in the vascular dysfunction that is a key mechanism in the development and progression of a wide range of cardiovascular diseases (Camussi G. et al., 2010). 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 (Horstman L.L. et al., 2004; Jesel L. et al., 2013; Lovren F., Verma S., 2013). Circulating CD31⁺/annexin V⁺ MPs affect both pro-inflammatory and pro-atherosclerotic processes, promote coagulation and inflammation, and also modulate angiogenesis and apoptosis in endothelial cells (Lovren F., Verma S., 2013; Montoro-García S. et al., 2013; Ohtsuka M. et al., 2013). Because endothelium is one of the primary targets of circulating microvesicles, 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 (Sinning J.M. et al., 2011). 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/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/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 emphasize 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 (Budaj M. et al., 2012; Barteneva N.S. et al., 2013; Mullier F. et al., 2013). There are data elucidated that a large pool of nanoparticles is produced after blood sampling due to fragmentation of blood cells (Suštar V. et al., 2011). 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.

Conflict of interests

Not declared

Ethical principles

The study was carried out in conformity with the Declaration of Helsinki.

Study Restrictions

This study has some restrictions. The author believes 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. I believe that these risks are systemic, and to minimize them, author refused to freeze the blood samples before measurement of

MPs. The authors suppose that these restrictions might have no significant impact on the study data interpretation.

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Прогностична цінність циркулюючих апоптотичних мікрочастинок у хворих з ішемічною маніфестною хронічною серцевою недостатністю

О.О. Крємзер

Резюме. Мета. Оцінити прогностичне значення циркулюючих CD31⁺/аннексин V⁺-мікрочастинок для кумулятивної виживаності у хворих з ішемічною хронічною серцевою недостатністю (ХСН). **Методи.** Обстежено 154 пацієнти з ішемічною ХСН

протягом 3 років після госпіталізації внаслідок прогресування захворювання. Зразки крові для наступного визначення рівня біомаркерів були зібрані однократно на початку дослідження. Фенотипування популяції мікрочастинок здійснювали методом проточної цитофлуориметрії за допомогою моноклональних антитіл, мічених флуорохромами FITC (флуоресцеїн ізотиоціанат) або подвійною міткою FITC/PE (фікоеритрин) для CD31⁺- та аннексин V⁺-антигенів. **Результати.** За період дослідження (медіана спостереження становила 2,18 року) було зареєстровано 21 смертельний випадок та 106 повторних госпіталізацій внаслідок ХСН. Медіани циркулюючого рівня CD31⁺/аннексин V⁺-мікрочастинок у пацієнтів з ХСН, що вижили або померли, були 0,286/мл (95% довірчий інтервал [ДИ]=0,271–0,309/мл) та 0,673/мл (95% ДИ=0,65–0,74/мл) ($p<0,001$). Кількість циркулюючих мікрочастинок було розподілено на квартилі (Q): Q1 (<0,341/мл), Q2 (0,342–0,514/мл), Q3 (0,521–0,848/мл) і Q4 (>0,850/мл). Аналіз отриманих даних показав значне розходження кривих виживаності Каплана – Мейера у пацієнтів з високим квартилем вмісту мікрочастинок з фенотипом CD31⁺/аннексин V⁺ (>0,514/мл) порівняно з більш низькими квартилями. При цьому кількість CD31⁺/аннексин V⁺-мікрочастинок та її поєднання із концентрацією N-термінального фрагменту промозгового натрійуретичного пептиду були статистично значущими незалежними предикторами для всіх смертельних випадків, смерті внаслідок ХСН, а також госпіталізацій, пов'язаних із прогресуванням ХСН. **Висновок.** Підвищення циркулюючого пулу мікрочастинок з фенотипом CD31⁺/аннексин V⁺ асоціюється з погіршенням 3-річної виживаності пацієнтів з ХСН.

Ключові слова: апоптотичні мікрочастинки, хронічна серцева недостатність, прогноз.

Прогностическая ценность циркулирующих апоптотических микрочастиц у больных с ишемической манифестной хронической сердечной недостаточностью

А.А. Крємзер

Резюме. Цель. Оценить прогностическое значение циркулирующих CD31⁺/аннексин V⁺-микро

частиц для кумулятивной выживаемости у больных с ишемической хронической сердечной недостаточностью (ХСН). **Методы.** Обследовано 154 пациента с ХСН на протяжении 3 лет после госпитализации, связанной с прогрессирующим заболеванием. Образцы крови для последующего определения уровня биологических маркеров отбирали однократно в начале исследования. Фенотипирование популяций микрочастиц осуществляли методом проточной цитофлуориметрии с помощью моноклональных антител, меченных флуорохромами FITC (флуоресцеин изотиоцианат) или двойной меткой FITC/PE (фикоэритрин) для CD31⁺- и аннексин V⁺-антигенов. **Результаты.** За время исследования (медіана наблюдения составила 2,18 года) зарегистрировано 21 смертельный исход и 106 повторных госпитализаций вследствие ХСН. Медианы циркулирующего уровня CD31⁺/аннексин V⁺-микрочастиц у выживших и умерших пациентов с ХСН составили 0,286/мл (95% доверительный интервал [ДИ]=0,271–0,309/мл) и 0,673/мл (95% ДИ=0,65–0,74/мл) ($p<0,001$). Количество циркулирующих микрочастиц было распределено на квартили (Q): Q1 (<0,341/мл), Q2 (0,342–0,514/мл), Q3 (0,521–0,848/мл) и Q4 (>0,850/мл). Анализ полученных данных показал значительное расхождение кривых выживаемости Каплана – Мейера у пациентов с высоким квартилем содержания микрочастиц (>0,514/мл) по сравнению с более низкими квартилями. При этом количество CD31⁺/аннексин V⁺-микрочастиц и их сочетание с концентрацией N-терминального фрагмента промозгового натрийуретического пептида являлись статистически значимыми независимыми предикторами для всех случаев смертельного исхода, смерти вследствие ХСН, а также госпитализаций, связанных с прогрессирующим ХСН. **Вывод.** Увеличение циркулирующего пула CD31⁺/аннексин V⁺-микрочастиц ассоциируется с ухудшением 3-летней выживаемости пациентов с ХСН.

Ключевые слова: апоптотические микрочастицы, хроническая сердечная недостаточность, прогноз.

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