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The predictive role of circulating microparticles in patients with chronic heart failure ☆

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ABSTRACT

Aim: The study aim was to evaluate whether circulating microparticles with apoptotic or non-apoptotic phenotypes are useful for risk assessment of 3-year cumulative fatal and non-fatal cardiovascular events in CHF patients.

Methods: The incidence of fatal and non-fatal cardiovascular events, as well as the frequency of occurrence of death from any cause in a cohort of 388 patients with CHF during 3 years of observation was studied prospectively. Circulating levels of NT-pro brain natriuretic peptide (NT-pro-BNP), high-sensitivity C-reactive protein (hs-CRP), and endothelial apoptotic microparticles (EMPs) were measured at baseline.

Results: Median follow-up was 2.32 years (IQR = 1.8–3.1). During follow-up, 110 cardiovascular events (including 43 fatal cases) were determined. Additionally, 74 subjects were hospitalized repetitively due to worsening CHF and also 16 subjects were readmitted in the hospital due to other cardiovascular reasons. In the univariate logistic regression analysis, the main factors independently related with cumulative endpoints were creatinine, fasting glucose, HbA1c, total cholesterol, uric acid, various types of EPMs, NT-pro-BNP, hs-CRP, NYHA class, decreased left ventricular ejection fraction (LVEF) less 45%, and type 2 diabetes mellitus. In multivariate model NYHA class, decreased LVEF (less 45%), NT-pro-BNP, hs-CRP, CD144 +/CD31 +/annexin V + EMPs, and CD31 +/annexin V + EMPs remained statistically significant for cumulative endpoint. Adding of CD144 +/CD31 +/annexin V + EMCs and CD31 +/annexin V + EMCs to the standard ABC model may improve the relative IDI for cumulative endpoint by 11.4% and 10.5% respectively.

Conclusion: Apoptotic phenotype of circulating microparticles may relate 3-year combined clinical outcomes in CHF patients.

1. Introduction

Chronic heart failure (CHF) remains a leading cause of cardiovascular morbidity and mortality [1]. Moreover, the frequencies of novel cases of CHF arise progressively worldwide [2]. Although the endothelium is considered an important target for traditional risk factors and endothelial dysfunction remained independently associated with mortality from CHF [3,4], the innate molecular mechanisms affected forming endothelial dysfunction are not fully clear. In this context, systemic pro-inflammatory activation, metabolic comorbidities, and neurohumoral state are considered the origin of microvascular endothelial cell inflammation that leads to the development of CHF and supports cardiac remodeling and vascular dysfunction [4]. Moreover, endothelial dysfunction is suggested as an early event in the development and progression of CHF. However, biological markers of endothelial dysfunction are abundant; there remains no ideal indicators that relate to the several faces of the pathogenesis of CHF [5,6]. The European Society of Cardiology and the American Heart Association/American Colleges of Cardiology have recently published a set of current biomarkers with high predictive value and powerful diagnostic capacity for subjects with CHF, which include natriuretic peptides, cardiac specific troponin, galectin-3, and high-sensitivity C-reactive protein [7–9]. Although all these markers have obvious advantages and some disadvantages too, there are several limitations regarding interpretations and implementation of obtained findings in risk stratification among CHF patients [10]. Collectively, discovering new biomarkers that closely

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related with nature evolution of CHF and tightly reflected different stages of disease appears to be attractive. Taking into consideration the pathogenesis of CHF and the role of endothelial dysfunction in nature evolution of cardiac failure, small-size cell membrane vesicles, such as microparticles, that are derived from activated cells or apoptotic particles, might be useful for risk stratification of the subjects with CHF. Indeed, wide spectrum endothelial cell-, platelet-, and monocyte/macrophage-derived microparticles have recently been associated with cardiovascular and metabolic diseases, inflammatory state, and autoimmune conditions.

Endothelial-derived microparticles (EMPs) are a novel biological marker of endothelial injury and vascular tone disorders [11,12]. EMPs are defined as a heterogeneous population of vesicles (diameter of 100–1000 nm) that are released by cellular vesiculation and fission of the membrane of endothelial cells [13]. EMPs derive from activated or apoptotic endothelial cells and may play a pivotal role in the vascular remodeling and endothelial reparation [14,15]. Biological effect of EMPs may mediate by supporting cell-to-cell cross-talking because EMPs transport miRNA, active molecules, hormones, peptides, regulator proteins, etc. [16,17]. Probably, EMPs may contribute to hyperadrenergic state in CHF via regulation of adrenal signaling [18,19]. However, knowledge on EMPs is sufficiently limited due to their submicrometer size and to intrinsic limitations in methods applied for their determination, while their role in CHF is probably underestimated. The study aim was to evaluate whether circulating endothelial-derived microparticles with apoptotic or non-apoptotic phenotypes are useful for risk assessment of 3-year cumulative fatal and non-fatal cardiovascular events in CHF patients.

2. Methods

2.1. Study population

The study population consisted of 388 consecutive patients with CHF who underwent angiography or PCI between April 2010 and June 2014, as well as referred as post-myocardial infarction subjects within this period in the five centers which participated in this investigation. All these patients were selected from 1427 patients according to our inclusion and exclusion criteria. The study protocol was approved by the Zaporozhye State Medical University ethics committee review board. The study complied with the Declaration of Helsinki and voluntary informed written consent was obtained from all patients included in this study.

Prognosis was assessed by the composite endpoint related to all-cause death, CHF-related death or CHF hospitalization, censored at 3 years.

2.2. 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. 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, and the procedure is available for assay. The coronary artery wall structure was measured by contrast-enhanced spiral computed tomography angiography [20] on Somatom Volume Zoom scanner (Siemens, Erlangen, Germany) with two detector rows using non-ionic contrast Omnipaque (Amersham Health, Ireland).

2.3. Echocardiography and tissue Doppler imaging

Transthoracic B-mode echocardiography and tissue Doppler imaging were performed according to a conventional procedure on ACUSON scanner (SIEMENS, Germany) using phased transducer of 5 MHz. Left ventricular end-diastolic and end-systolic volumes, and ejection fraction (LVEF) were measured by modified Simpson's planimetric method [21,22]. Inter- and intra-observer variability coefficients for LVEF were 3.2% and 1.1% respectively.

2.4. Glomerular filtration rate measurement

Calculation of glomerular filtration rate (GFR) was calculated by CKD-EPI formula [23].

2.5. Biomarker determination

All biomarkers were determined at baseline. To measurement of biological marker concentrations, blood samples were drawn 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 6000 rpm for 3 min. Then, plasma was refrigerated immediately to be stored at a temperature −70 °C until measurement.

Circulating NT-pro-BNP level was measured by immunoelectrochemoluminescent assay using sets produced by R&D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). The high-sensitivity C-reactive protein (hs-CRP) levels were measured by using nephelometric technique on AU640 analyzer manufactured by Diagnostic Systems Group (Japan).

Concentrations of total cholesterol (TC) and cholesterol of high-density lipoproteins (HDLP) were measured by the fermentation method. Concentration of cholesterol of low-density lipoproteins (LDL-C) was calculated according to the Friedewald formula (1972).

A total of 100 μL of serum samples was assayed in parallel to known standard concentrations for each biological marker. The mean intra-assay coefficients of variation were <10% of all cases.

2.6. Endothelial-derived apoptotic and activated microparticle determination

Endothelial-derived apoptotic and activated microparticles were phenotyped by flow cytometry by phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (platelet endothelial cell adhesion molecule [PECAM]-1), CD144 (vascular endothelial [VE]-cadherin), CD62E (E-selectin), and annexin V (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). For determination of annexin V+ EMPs 400 μL annexin V binding buffer was added. For each sample, 500,000 events have been analyzed. EMPs gate was defined by size, using 0.8 and 1.1 mm beads (Sigma, St. Louis, MO, USA). CD31+/annexin V+ and CD144+/CD31+/annexin V+ microparticles were defined as apoptotic EMPs, EMPs positively labeled for CD62E+ were determined as EMPs produced due to activation of endothelial cells. Therefore, double-positive EMPs (CD31 and CD144) and triple-positive (CD144+/CD31+/annexin V+) were defined as most specific EMPs [24,25].

2.7. Statistical analysis

Statistical analysis of the results obtained was carried out in SPSS system for Windows, Version 22 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism for Windows, Version 5 (GraphPad Software Inc., La Jolla, CA, USA). The data were presented as mean (M) and standard

deviation (±SD) or 95% confidence interval (CI); median (Me) and interquartile range (IQR), as well as number (*n*) and frequencies (%) for categorical variables. To compare the main parameters of patients' groups (subject to the type of distribution of the parameters analyzed), two-tailed Student's *t*-test or Mann–Whitney *U*-test was used. To compare categorical variables between groups, chi-squared test ($\chi 2$) and Fisher *F* exact test were used. The factors, which could be associated potentially with clinical outcomes, were determined by log regression analysis. Reclassification methods (*C*-statistics) were utilized for prediction performance analyses. A calculated difference of $P < 0.05$ was considered significant.

3. Results

3.1. Study patient population

The characteristics of the patients participated in the study are depicted in Table 1. At baseline, mean age in box sexes was 58.34 years. The prevalence of II (37.9%) and III (21.4%) NYHA class was determined. At least 55.5% of the subjects enrolled in the study were hypertensive. Likewise, cardiovascular risk factors, such as dyslipidemia, type 2 diabetes mellitus and obesity, were reported 66.0%; 37.6%; and 44.3% respectively. Mean left ventricular ejection fraction was decreased slightly. Subjects who experienced the composite endpoint presented frequently III–IV NYHA classes, lower estimated glomerular filtration ratio, as well as higher uric acid, creatinine level, NT-pro-BNP, hs-CRP, and lipid abnormalities.

The majority of patients with CHF were treated with ACE inhibitors or ARAs, beta-adrenoblockers, I/f blocker ivabradine, mineralocorticoid receptor antagonists, and antiplatelet drugs (Table 2). Adding loop diuretics was done when fluid retention was determined. Dihydropyridine calcium channel blockers were added to hypertensive subjects when blood pressure was not controlled by previous treatment scheme. Metformin and/or sitagliptin were used in type 2 diabetes patients as a component of contemporary treatment of CHF. Loop diuretics and aspirin were prescribed frequently in subjects who experienced the composite endpoint. In opposite, beta-adrenoblockers, statins and sitagliptin were given frequently among patients who did not.

3.2. Clinical event determination

Median follow-up was 2.32 years (IQR = 1.8–3.1). During follow-up, 110 cardiovascular events (including 43 fatal cases) were determined. Thirty five patients died due to advance of CHF, and eight cases of death were related with sudden death, fatal myocardial infarction, and systemic thromboembolism. No other causes of death were defined. Additionally, 74 subjects were readmitted after discharge from the hospital due to worsening CHF and 16 subjects were readmitted due to other cardiovascular reasons.

3.3. Microparticles in CHF patients

As shown in Fig. 1A, total numbers of CD144+/annexin V+ phenotyped endothelial-derived microparticles (EMPs) were not different between both patient cohorts ($P = 0.22$). A double-positive for CD144 and CD31-staining phenotype of EMPs showed a significant increase in patients who experienced the composite endpoints when compared with those who did not (Fig. 1B). Therefore, Fig. 1C presented that CD144+/CD31+/annexin V+ EMPs were too elevated significantly in subjects who met combined endpoints when compared with patients who did not ($P = 0.001$). Mean of circulating apoptotic EMP numbers (CD31+/annexin V+) in patients who experienced the composite endpoints was higher when compared with mean of EMPs isolated from peripheral blood from patients who did not ($P < 0.001$) (Fig. 1D). In contrast, activated CD62E+ EMP numbers were not significantly different between the patient's cohorts ($P = 0.46$) (Fig. 1F).

Table 1
The characteristics of participants.

	Entire patient cohort ($n = 388$)	Subjects who experienced the composite endpoint ($n = 110$)	Subjects who did not experience the composite endpoint ($n = 278$)	*P* value
Age, years	58.34 ± 9.60	57.32 ± 6.15	58.73 ± 7.22	0.86
Male, *n* (%)	207 (53.3%)	64 (58.2%)	143 (51.4%)	0.88
I NYHA class, *n* (%)	77 (19.8%)	–	77 (27.7%)	0.001
II NYHA class, *n* (%)	147 (37.9%)	26 (23.6%)	121 (43.5%)	0.001
III NYHA class, *n* (%)	83 (21.4%)	52 (47.3%)	31 (11.2%)	0.001
IV NYHA class, *n* (%)	81 (20.9%)	32 (29.1%)	49 (17.6%)	0.001
Hypertension, *n* (%)	214 (55.5%)	62 (56.4%)	152 (54.7%)	0.96
Dyslipidemia, *n* (%)	256 (66.0%)	48 (43.6%)	208 (74.8%)	0.024
Type 2 diabetes mellitus, *n* (%)	146 (37.6%)	42 (38.2%)	104 (37.4%)	0.94
Obesity, *n* (%)	172 (44.3%)	54 (49.1%)	118 (42.4%)	0.82
Adherence to smoke, *n* (%)	76 (19.6%)	25 (22.7%)	51 (18.3%)	0.77
BMI, kg/m^2	24.1 (95% CI = 21.6–28.7)	23.9 (95% CI = 20.7–25.9)	23.3 (95% CI = 21.5–24.8)	0.68
Systolic BP, mm Hg	131 ± 8	130 ± 5	133 ± 5	0.84
Diastolic BP, mm Hg	78 ± 5	77 ± 4	78 ± 4	0.92
Heart rate, beats per min	70.52 ± 3.34	74.60 ± 4.6	69.10 ± 6.2	0.48
LVEF, %	42.80 ± 5.76	42.20 ± 3.11	43.20 ± 6.18	0.76
GFR, 1.73 mL/min/m^2	82.3 (95% CI = 68.7–102.6)	81.5 (95% CI = 71.3–94.7)	83.9 (95% CI = 77.1–102.6)	0.055
Creatinine, μmol/L	72.3 (95% CI = 58.7–92.6)	73.1 (95% CI = 60.9–80.5)	70.7 (95% CI = 59.1–88.1)	0.048
Fasting glucose, mmol/L	5.20 (95% CI = 3.3–9.7)	5.27 (95% CI = 3.5–9.4)	4.98 (95% CI = 3.8–8.1)	0.28
HbA1c, %	6.8 (95% CI = 4.1–9.5)	6.9 (95% CI = 4.3–9.2)	6.6 (95% CI = 4.6–8.3)	0.36
Hemoglobin, g/L	135.4 (95% CI = 128.5–140.1)	134.1 (95% CI = 126.2–136.4)	136.1 (95% CI = 125.1–144.8)	0.06
Total cholesterol, mmol/L	5.1 (95% CI = 3.9–6.1)	5.3 (95% CI = 4.6–6.0)	5.0 (95% CI = 3.5–5.9)	0.047
Cholesterol HDL, mmol/L	0.91 (95% CI = 0.89–1.12)	0.96 (95% CI = 0.93–1.05)	0.88 (95% CI = 0.84–1.01)	0.044
Cholesterol LDL, mmol/L	3.23 (95% CI = 3.11–4.40)	3.71 (95% CI = 3.50–4.20)	3.53 (95% CI = 3.11–3.97)	0.06
Uric acid, mmol/L	33.5 (95% CI = 25.3–40.1)	35.7 (95% CI = 25.3–40.1)	31.1 (95% CI = 20.6–36.9)	0.036
NT-pro-BNP, pg/mL	1977.2 (95% CI = 984.7–2993.2)	2616.5 (95% CI = 1085.3–3683.5)	1530.6 (95% CI = 644.5–2560.6)	0.042
hs-CRP, mg/L	7.34 (95% CI = 6.77–7.95)	8.04 (95% CI = 6.81–9.52)	6.96 (95% CI = 5.03–8.13)	0.036

Notes: *P* value was calculated between variables for subjects who experienced the composite endpoint and who did not; data were presented as median and 95 confidence interval (CI); NYHA — New York Heart Association; GFR — glomerular filtration rate; BMP — brain natriuretic peptide; BP — blood pressure; LVEF — left ventricular ejection fraction; BMI — body mass index, EMPs — endothelial-derived apoptotic microparticles; HbA1c — glycated hemoglobin, HDL — high-density lipoprotein; LDL — low-density lipoprotein.

Table 2
Treatment strategy in CHF patients enrolled in the study.

	Entire patient cohort ($n = 388$)	Subjects who experienced the composite endpoint ($n = 110$)	Subjects who did not experience the composite endpoint ($n = 278$)	*P* value
ACE inhibitors or ARAs, *n* (%)	388 (100%)	110 (100%)	278 (100%)	1.0
Aspirin, *n* (%)	305 (78.6%)	96 (87.3%)	209 (75.2%)	0.022
Other antiplatelet drugs, *n* (%)	83 (21.4%)	14 (12.7%)	69 (24.8%)	0.026
Beta-adrenoblockers, *n* (%)	324 (83.5%)	73 (66.4%)	251 (90.3%)	0.001
Dihydropyridine calcium channel blockers, *n* (%)	63 (16.2%)	17 (15.5%)	46 (16.5%)	0.88
Ivabradine, *n* (%)	137 (35.3%)	43 (39.0%)	94 (33.8%)	0.78
Mineralocorticoid receptor antagonists, *n* (%)	152 (39.2%)	45 (40.9%)	107 (38.5%)	0.66
Loop diuretics, *n* (%)	311 (80.1%)	110 (100%)	201 (72.3%)	0.043
Statins, *n* (%)	294 (75.7%)	48 (43.6%)	246 (88.5%)	0.012
Metformin, *n* (%)	146 (37.6%)	42 (38.2%)	104 (37.4%)	0.86
Sitagliptin, *n* (%)	48 (12.4%)	9 (8.2%)	40 (14.4%)	0.001

Notes: *P* value was calculated between variables for subjects who experienced the composite endpoint and who did not; data were presented as number and frequency; ACE — angiotensin-converting enzyme; ARAs — angiotensin-2 receptor antagonists.

Close association between total numbers of CD144+/annexin V+ EMPs with NYHA class ($r = 0.41$, $P = 0.002$), NT-pro-BNP ($r = 0.31$, $P = 0.001$), and LVEF ($r = -0.26$, $P = 0.001$) was found. The data shown that CD144+/CD31+ EMP numbers were positively associated with NYHA class ($r = 0.52$, $P = 0.001$), NT-pro-BNP ($r = 0.44$, $P = 0.003$), uric acid ($r = 0.40$, $P = 0.001$), hs-CRP (0.33, $P = 0.001$), and T2DM ($r = 0.32$, $P = 0.003$), and inversely associated with LVEF ($r = -0.32$, $P = 0.001$), and HDL cholesterol ($r = -0.221$, $P = 0.002$). EMP labeled as CD31+/annexin V+ was positively associated with NYHA class ($r = 0.73$, $P = 0.001$), NT-pro-BNP ($r = 0.689$, $P = 0.001$), uric acid ($r = 0.41$, $P = 0.001$), hs-CRP (0.408, $P = 0.006$), and T2DM ($r = 0.402$, $P = 0.003$), and inversely associated with LVEF ($r = -0.496$, $P = 0.001$), eGFR ($r = -0.408$, $P = 0.003$), and HDL cholesterol ($r = -0.221$, $P = 0.002$). There was a significant association between CD62E+ EMP number and uric acid ($r = 0.312$, $P = 0.001$), hs-CRP (0.302, $P = 0.001$), and HDL cholesterol ($r = -0.26$, $P = 0.001$). No significant association between the levels of circulating EMPs with fasting plasma glucose, HbA1c, means systolic and diastolic blood pressure (BP), and medications for both cohorts of the patients was found.

3.4. Predictors of cumulative cardiovascular events

Univariate and multivariate logistic regression were used to assess whether any biomarker was able to predict better in CHF patients (subjects who experienced the composite endpoint versus who did not). Univariate logistic regression analysis shown that the main factors independently related with cumulative endpoints were creatinine, fasting glucose, HbA1c, total cholesterol, serum uric acid, various types of EPMs, NT-pro-BNP, hs-CRP, NYHA class, decreased LVEF (less 45%), and T2DM (Table 3). In multivariate model NYHA class, LVEF less 45%, NT-pro-BNP, hs-CRP, CD144+/CD31+/annexin V+ EMPs, and CD31+/annexin V+ EMPs remained statistically significant for cumulative endpoint.

We used reclassification methods aimed to define whether the addition of EMCs labeled as CD144+/CD31+/annexin V+ and CD31+/annexin V+ to the standard ABC model constructed with NYHA class, decreased LVEF (less 45%), NT-pro-BNP, and hs-CRP to improve the discriminate value of the model. The analysis of obtained result showed that adding CD144+/CD31+/annexin V+ EMCs and CD31+/annexin V+ EMCs to the standard ABC model may improve the relative IDI for cumulative endpoint by 11.4% and 10.5% respectively. For category-free NRI, 5% of events ($P = 0.003$) and 6% of non-events ($P = 0.001$) were correctly reclassified by the addition of CD144+/CD31+/annexin V+ EMCs to the ABC model. When we added CD31+/annexin V+ EMCs to the ABC model, 3% and 4% of events ($P = 0.001$ for all cases) for category-free NRI and 6% and 7% of non-events ($P = 0.001$ for all cases) were correctly reclassified. Thus, apoptotic phenotype of circulating EMPs may relate to 3-year combined clinical outcomes in CHF patients.

4. Discussion

This study demonstrates that EMPs obtained from peripheral blood in CHF patients may contribute in clinical outcomes. However, EMP patterns in patients who experienced cumulative events and subjects who did not were different. Predominantly apoptotic profile of circulating EMPs was presented through CD144+/CD31+/annexin V+ EMPs and CD31+/annexin V+ EMPs. The numbers of apoptotic EMPs in patients who met cumulative clinical outcomes were significantly higher when compared with those who did not. No sufficient difference between both patient cohorts regarding numbers of EMPs appeared to be in peripheral blood in resulting activation of the endothelial cells. Therefore, there was lack of significant difference in low specific EMPs labeled as CD144+/annexin V+ microparticles in patients who experienced cumulative clinical outcomes and those who did not. Overall, these findings mirror that apoptotic EMP pattern appears to be negatively prognostically potent for CHF patient population. Therefore, it is shown that the EMP profile may be considered a useful tool for determination of the CHF patients with poor prognosis. Indeed, adding of CD144+/CD31+/annexin V+ EMCs and CD31+/annexin V+ EMCs to the standard ABC model that was constructed with contemporary predictive biomarkers, including NYHA class, decreased LVEF (less 45%), NT-pro-BNP, and hs-CRP, allows to improve the discriminate value of the standard model.

Because EMPs are considered a mediator for inflammatory responses that may contribute in vascular wall injury [26], the results of the study support the hypothesis about the pivotal role of endothelial dysfunction in worsening of CHF. Recent investigations show that various stimuli, such as shear stress, inflammation, and vascular damage may potentiate to realize EMPs by two leading ways: via secretion from activated endothelial cells and via induction of endothelial cell apoptosis [27,28]. As shown circulating EMPs derived from activated endothelial cells play a pivotal role in tissue repair, transcellular exchange of information, and neoangiogenesis [29]. In opposite, apoptotic-derived EMPs contribute in vascular damage through stimulation of tissue factor-dependent procoagulant activity, free-radical hyper-production and inflammation, leukocyte adhesion, and altered vasomotion [30–33]. Biological effects of EMPs realize via the induction of the specific cellular properties that leads to activation of membrane-associated receptors on the surface of wide spectrum of target cells, including endothelial cells, and mononuclears [34]. We suggested that predominantly elevated apoptotic EMPs create opportunity for reprogramming and phenotype modification of endothelial cells that lead to worsening of endothelial dysfunction and as a result in poor

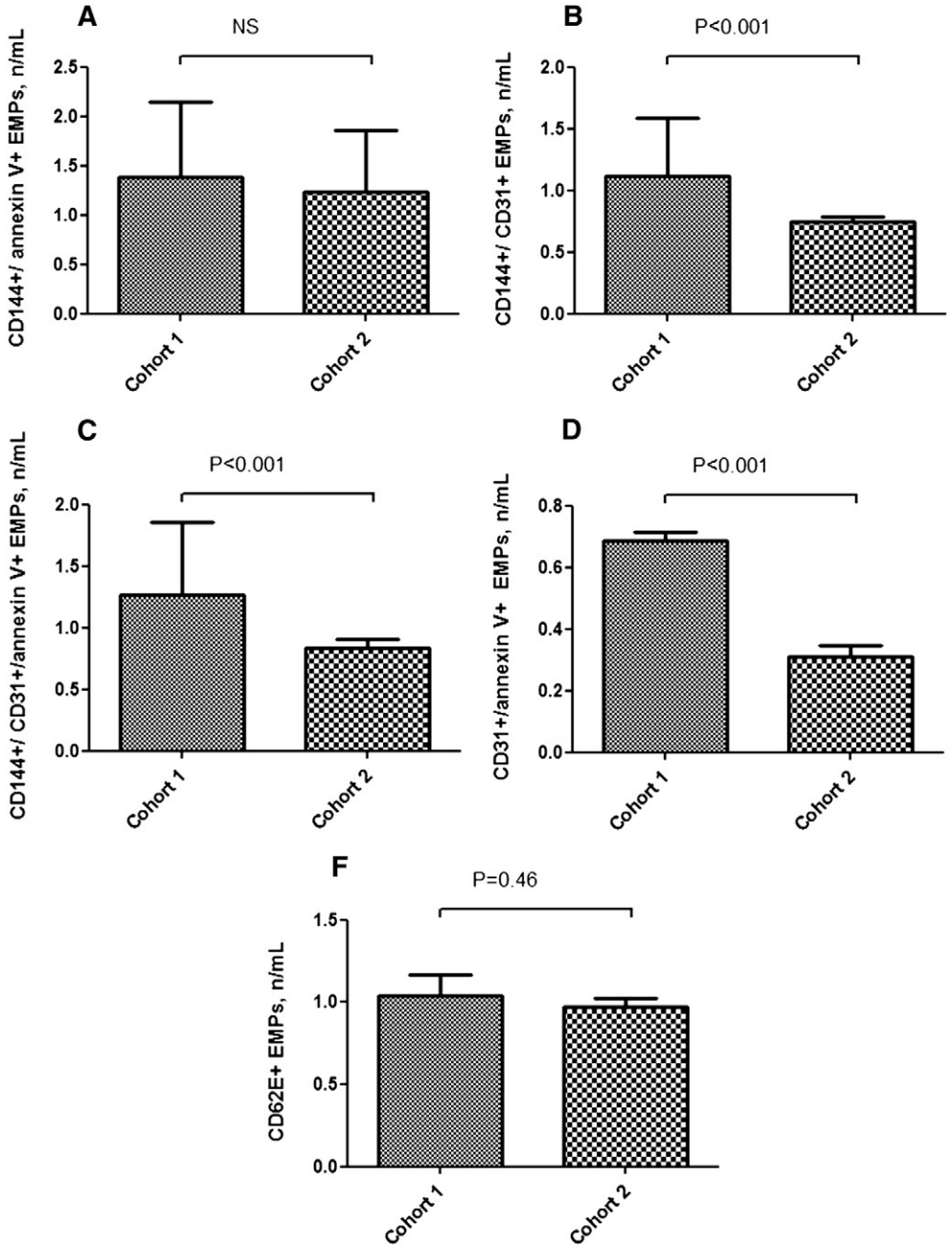

Fig. 1. Comparison of the profile of circulating endothelial-derived microparticles in patients who experienced the composite endpoint (Cohort 1) and who did not (Cohort 2). Values for all figures are mean and SD (standard deviation) and were compared using Student's paired *t*-test. A. CD144 + /annexin V + EMP numbers isolated from peripheral blood from both cohort patients. B. Circulating CD144 + /CD31 + EMPs in both cohort patients. C. CD144 + /CD31 + /annexin V + EMP numbers isolated from circulation from both cohort patients. D. CD31 + /annexin V + EMP numbers isolated from peripheral blood from both cohort patients. F. CD62E + EMP numbers isolated from peripheral blood from both cohort patients.

clinical outcomes. Although apoptotic phenotypes of EMPs are not specific for CHF only and may determine in other cardiovascular and metabolic diseases [35,36], the exact innate mechanisms affecting predominantly elevation of apoptotic-derived EMPs in CHF are still not understood and required detailed investigations. In fact, the patients with a higher EMP level had a higher risk of future cardiovascular events including major adverse cardiovascular events, recurrent hospitalizations and death [34–36]. Moreover, the interrelation between peripheral endothelial dysfunction and cardiovascular events at high-risk patients may contribute in realizing the apoptotic phenotype of EMPs [37,38]. The results of our investigations showed that numbers of apoptotic-derived EMPs better predict clinical outcomes in CHF patients than activated endothelial cell-derived microparticle pattern. In support of this issue we have recently reported that increased apoptotic EMPs to progenitor mononuclear cell ratio was a better predictor when compared with isolated decreased proangiogenic progenitor mononuclears, which are suitable for patients with CHF [39]. Thus, apoptotic phenotype of EMPs is essential for cardiac failure subjects with short-term poor outcomes. These data are very promising, and they require a new investigation with higher statistical power and increased sample size to overcome the internal limitations of the study.

In conclusion, we suggest that apoptotic phenotype of EMPs labeled as CD144 + /CD31 + /annexin V + and CD31 + /annexin V + may be considered as a useful tool for determination of CHF patients with

Table 3
Univariate and multivariate regression analyses.

Variances	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
NYHA class	1.15	1.03–1.29	0.006	1.06	1.01–1.09	0.001
T2DM (present vs. absent)	1.05	1.01–1.09	0.006	1.03	0.89–1.07	0.001
LVEF less 45% (present vs. absent)	1.56	1.23–1.72	0.002	1.17	1.04–1.25	0.003
Creatinine per 30 μmol/L	1.06	1.01–1.11	0.001	1.02	0.87–1.06	0.001
Fasting glucose per 3 mmol/L	1.04	0.96–1.09	0.002			
HbA1c per 1%	1.05	1.01–1.07	0.002			
Total cholesterol per 1 mmol/L	1.08	1.01–1.09	0.001			
Uric acid per 10 mmol/L	1.08	1.03–1.09	0.001	1.03	0.92–1.08	0.001
NT-pro-BNP per 400 pg/mL	1.97	1.25–3.06	0.001	1.37	1.08–2.10	0.001
hs-CRP per 1 mg/L	1.32	1.22–1.57	0.001	1.12	1.03–1.25	0.001
CD144 +/annexin V + EMPs per 0.3/mL	1.02	1.00–1.04	0.001	0.96	0.89–1.10	0.001
CD144 +/CD31 + EMPs per 0.5/mL	1.04	1.02–1.06	0.001	1.02	0.94–1.05	0.001
CD144 +/CD31 +/annexin V + EMPs per 0.6/mL	1.34	1.18–1.62	0.006	1.19	1.12–1.33	0.001
CD31 +/annexin V + EMPs per 0.2/mL	1.18	1.10–1.27	0.001	1.07	1.02–1.13	0.001
CD62E + EMPs per 0.15/mL	1.03	1.00–1.08	0.001	0.99	0.94–1.05	0.001

Notes: CI — confidence interval; OR — odds ratio; HbA1c — glycated hemoglobin; BNP — brain natriuretic peptide; EMPs — endothelial-derived apoptotic microparticles.

poor prognosis. Finally, identification of the pattern of circulating EMPs may help to determine patients at high risk and reclassify it for possible biomarker-guided therapy of CHF.

5. Study limitations

This study has some limitations. It is necessary to note that a large pool of nanoparticles might be produced after blood sampling due to destruction of platelets and blood cells. Therefore, preparation of isolates of microparticles in the samples is the most sophisticated step for further examination. Venous citrated blood drawn from the fistula-free arm was performed obligatorily. We believe that these risks are systemic, and to minimize them, we refused to freeze the blood samples before measurement of microparticles. Therefore, there were several technical-related difficulties in the measurement of EMPs. In fact, there was a lack of standard protocol for isolating and detecting circulating EMPs obtained from the plasma. According to the opinion of the majority of experts, centrifugation has become the main factor mediated reliability of the EMP determination in samples and contributed in biological variability of EMP count. Although HD-FACS methodology is widely used, theoretically overlap between two or more fluorochromes might reflect some obstacles for further interpretation of obtained results. Another limitation of the present study is that a specific role of EMPs is also possible and has not been characterized in depth in CHF patients especially with various comorbidities. However, the authors suppose that these restrictions might have no significant impact on the study data interpretation. Additionally, retrospective, relative small sample size may limit the significance of the present study. However, this was not a randomized and controlled study. The authors believe that a greater cohort of patients with more incidences detected is desirable to improve the credibility of the study.

Conflict of interest

None declared.

Abbreviations

BMI body mass index
BMP brain natriuretic peptide
CI confidence interval
CHF chronic heart failure
EMPs endothelial-derived microparticles
GFR glomerular filtration rate
LVEF left ventricular ejection fraction
NYHA New York Heart Association
OR odds ratio

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Ethical principles

All the patients have given their voluntary written informed consent for participation in the study. The study was approved by the local ethics committee of the State Medical University, Zaporozhye, Ukraine. The study was carried out in conformity with the Declaration of Helsinki.

Authors' contributions

Alexander E. Berezin initiated the hypothesis and designed the study protocol, contributed to collect, analyze and interpret the data, performed statistical analysis, and wrote the manuscript. Alexander A. Kremzer contributed to enroll the patients, collected and analyzed the data, checked clinical events and reviewed the source documents. Yulia V. Martovitskaya contributed in circulating biomarker determination, performed preparation of isolates of microparticles in samples with further phenotyping by flow cytofluorometry, and interpreted the obtained results. Tatyana A. Samura preformed visualization procedures and analyzed the results of examinations. Tatyana A. Berezina contributed in enrolling the patients in the study and collecting the data.

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