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Original article

Predictive value of apoptotic microparticles to mononuclear progenitor cells ratio in advanced chronic heart failure patients☆

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ABSTRACT

Background: Acutely decompensated chronic heart failure (ADHF) is considered a life-threatening event. Despite contemporary treatment strategies of ADHF, frequent recurrent hospitalizations due to other cardiovascular reasons after discharge of patients from hospital occur. The objective of the study was to examine the prognostic value of circulating endothelial-derived apoptotic microparticles (EMPs) to mononuclear progenitor cells (MPCs) ratio for post-discharge patients with clinical stabilization after ischemic ADHF.

Methods: We consecutively enrolled 136 patients (62 male) with coronary artery disease (CAD) admitted with a primary diagnosis of ADHF. All patients gave written informed consent for participation in the study. At baseline, all enrolled patients were hemodynamically stable and they had New York Heart Association (NYHA) III/IV classes of ischemic chronic heart failure (CHF). Observation period started at discharge from the hospital and was up to 3 years. Flow cytometry analysis for quantifying the number of EMPs and angiogenic MPCs was used.

Results: Calculated EMP to MPC ratios in survivor and dead patient cohort were 8.4 (95% CI = 7.6–9.2) and 78.9 (95% CI = 53.0–116.6), respectively ($p = 0.001$). MPCs, EMPs, NYHA class, N-terminal pro-brain natriuretic peptide (NT-proBNP) and increased NT-proBNP > 30% within 24–84 h of admission period remained statistically significant for all-cause mortality, CHF-related death, and CHF-related rehospitalization, whereas left ventricular ejection fraction and high-sensitivity C-reactive protein for all variables did not. We found that the addition of EPMs to MPCs ratio to the ABC model (NT-pro-BNP, increased NT-pro-BNP > 30%) improved the relative integrated discrimination indices by 19.6% for all-cause mortality, by 21.7% for CHF-related death, and by 19.5% for CHF-related rehospitalization.

Conclusion: We demonstrated that EMP to MPC ratio is considered an important indicator of an imbalance between angiogenic and apoptotic responses with possible relation to cardiovascular outcomes in post-discharge patients with clinical stabilization after ischemic ADHF.

Introduction

Acutely decompensated chronic heart failure (ADHF) is considered a life-threatening event with a short-term in-hospital mortality range of 4.5–8.5% [1]. Despite contemporary treatment strategies for ADHF, frequent recurrent hospitalizations due to other cardiovascular reasons after discharge from hospital occur [2]. Recent investigations have shown that both neurohormonal and low-intensity proinflammatory activation play pivotal roles in cardiovascular remodeling, which leads to worsening of chronic heart failure (CHF) [3]. Therefore, there are exacerbating factors for advanced CHF

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and hospital readmission including acute coronary syndromes, infections, non-compliance with low-salt diet, and non-compliance with CHF medications [4]. However, each such episode of worsening CHF that usually requires hospitalization is associated with innate mechanisms of the development and deterioration of cardiovascular remodeling [5]. This phenomenon of mutual interrelationship of worsening CHF and cardiovascular remodeling due to other exacerbating factors is described as a "pathologic circle" [6]. Several biological factors that are independent predictors of morbidity and mortality in patients with advanced CHF have been found [7,8]. Indeed, changes in brain natriuretic peptide (BNP) levels during treatment of acutely decompensated CHF were strongly associated with early readmission and mortality in the short-term perspective [3,9]. Serial measurements of BNP, high-sensitivity C-reactive protein (hs-CRP), and cardiac-specific troponin in circulation among patients with CHF after discharge from hospital with the aim to assay the risk of acute or acutely decompensated myocardial dysfunction have been widely discussed [3,10,11]. Data on biological variation of BNP, hs-CRP and troponin I show the differences in results and individuality expected in CHF patients, suggesting that serial measurements need to be undoubtedly interpreted carefully [12,13]. All these initiate searching new biomarkers that have higher predictive value for CHF progression in the post-discharge period among surviving subjects with ADHF after achieving clinical stabilization.

It is now well established that a deterioration of vascular integrity and endothelial function, angiogenesis, coagulation, and inflammation represents a crucial event in the development of CHF [14,15]. Recent studies suggested that circulating level of both endothelial-derived apoptotic microparticles (EMPs) [16] and mononuclear progenitor cells (MPCs) with proangiogenic capacities are key players in the pathogenesis of cardiac failure [17]. EMPs are a heterogeneous population of submicronic vesicles that are released in response to cell activation or apoptosis [18]. MPCs ensure reparative processes including endothelialization of vascular lesion fragments, as well as remodeling of extracellular matrix and neovascularization [19]. MPCs, which co-expressed CD34+ antigen and VEGFR-2+ vascular growth ligands (vascular endothelial growth factor receptor-2), CD133+, CD14+, and Tie2+ (tyrosine kinase ligand), were tested as predictors of nature evolution of ischemic CHF [17]. We previously reported about perspective-in-use of new biomarker represented EMPs to MPCs ratio in subjects with stable mild-to-moderate CHF [20]. The objective of the study was to examine prognostic value of circulating EMPs to MPCs ratio for post-discharge patients with clinical stabilization after ischemic acutely decompensated CHF.

Methods

We consecutively enrolled 136 patients (both sexes, 62 male) with CAD admitted with a primary diagnosis of ADHF in the hospital between February 2010 and November 2012. All the patients gave their voluntary written informed consent for participation in the study. At baseline all enrolled patients were hemodynamically stable and they had NYHA III/IV classes of ischemic CHF. The observation period started at discharge from the hospital and was up to 3 years. We checked all deaths, including CHF-related death, sudden death, and all cases of repeat hospitalizations, including cases such as worsening CHF. Dyslipidemia, hypertension, and type 2 diabetes mellitus were defined according to contemporary clinical guidelines [21–23].

Methods for visualization of coronary arteries

Multispiral computed tomography angiography and/or angiographic studies have been carried out to verify the ischemic nature of the disease in patients. Multispiral computed tomography angiography was carried out for all the patients prior to their inclusion in the study after clinical stabilization and prior discharge from the hospital. 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 previously provided no new cardiovascular events occurred during this period, and the procedures were available for assay. The coronary artery wall structure was measured by means of contrast spiral computed tomography angiography [24] on Somatom Volum Zoom scanner (Siemens, Erlangen, Germany) with two detector rows when the patients were holding their breath at the end of inspiration. After preliminary native scanning, non-ionic contrast Omnipak (Amersham Health, Carrigtwohill, 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 echocardiographic examination

Transthoracic echocardiographic examination was performed according to a conventional procedure on all patients using an Acuson Sequola scanner (Siemens) in B-mode regimen and tissue Doppler echocardiography regimen from parasternal, subcostal, and apical positions over the short and long axes with a transducer with 5-mHz frequency along with electrocardiographic monitoring. Examination was performed by finding convenient echocardiographic windows when the patient was in the decubitus position or lying on their left side. 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 [25]. Doppler velocity curves were recorded at a horizontal sweep speed of 100 mm/s while the patients held their breath after exhalation. An average of 3 consecutive cycles was used for the analysis. Tissue Doppler echocardiography was carried out in 4-chamber view in each of 16 segments of the left ventricle and in 4 spots of the mitral annulus [26]. Peak systolic mitral annular (Sm), early diastolic (Em), and late diastolic (Am) myocardial velocities were measured with the sample volume placed at the junction of the LV wall and the mitral annulus of the septal and lateral myocardial segments from the 4-chamber view, 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 [27].

Measurement of NT-pro-BNP, hs-C-reactive protein, total cholesterol, and its fractions

Blood samples were taken from all patients from the antecubital vein for the measurement of N-terminal pro-brain natriuretic peptide (NT-pro-BNP), total cholesterol (TC) and cholesterol fractions, and hs-C-reactive protein. Blood samples taken in the morning (at 07.00–08.00 h) were drawn 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 not higher than −35 °C.

Circulating NT-pro-BNP level was measured at admission and at discharge of the patients. Assessment was performed by

electrochemiluminescent immunoassay method using R&D Systems (Minneapolis, MN, USA) commercial kits on Elecsys 1010 analyzer (Roche, Mannheim, Germany).

The hs-CRP levels were measured by using nephelometric technique on AU640 analyzer manufactured by Diagnostic Systems Group (Kobe, Japan).

Concentrations of 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 EMPs 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 centrifuged at 20,500 × rpm for 30 min. EMP pellets were washed with Dulbecco's minimal essential medium (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, EMPs, pellet, and supernatant were diluted 5-, 10-, and 5-fold in PBS, respectively. Endothelial-derived apoptotic microparticles were phenotyped by flow cytofluorimetry by phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (BD Biosciences, Franklin Lakes, NJ, USA) followed by incubation with fluorescein isothiocyanate (FITC)-conjugated Annexin V (BD Biosciences) per high-definition fluorescence activated cell sorter (HD-FACS) methodology independently after supernatant was diluted without freezing [28]. 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, Brea, CA, USA) after 400 L annexin-V binding buffer was added. For each sample, 500,000 events were analyzed. EMPs gate was defined by size, using 0.8 and 1.0 mm beads (Sigma, St. Louis, MO, USA). CD31+/Annexin V+ microparticles were defined as EMPs positively labeled for CD31 and Annexin V (CD31+/Annexin V+) [29].

Identifying fractions of mononuclear and endothelial progenitor cells

Mononuclear cell populations were phenotyped by flow cytofluorimetry by means of monoclonal antibodies labeled with fluorescein isothiocyanate (FITC) fluorochromes or double-labeled with FITC/phycoerythrin (PE) (BD Biosciences) to CD45, CD34, CD14, Tie-2, and CD309 (VEGFR2) antigens as per HD-FACS methodology, with red blood cells removed obligatory with lysing buffer according to gating strategy of International Society of Hematotherapy and Graft Engineering sequential (ISHAGE protocol of gating strategy) [30]. For each sample, 500,000 events were analyzed. Circulating MPCs have been identified as CD45−CD34+ cells. Proangiogenic phenotype for endothelial MPCs was determined as CD14+CD309 (VEGFR2)+Tie-2+ antigens. All data were obtained when laser beam was scattered in longitudinal and transversal directions in the flow cytofluorimeter, and the scattergram results were analyzed by using Boolean principles for double or triple positive events.

Statistical analysis

Statistical analysis of the obtained results was carried out in SPSS system for Windows, Version 22 (SPSS Inc., Chicago, IL, USA). The data were presented as mean (*M*) and standard deviation (±SD) or 95% confidence interval (CI), as well as median (Me) and interquartile range (IQR). 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 was used. To compare categorical variables between groups, Chi^2 test (χ^2) and Fisher *F* exact test were used. The circulating EMPs, MPCs, and NT-pro-BNP level in the blood failed to have a normal distribution, while distribution of the hs-CRP, 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 that could be associated potentially with EMP to MPC ratio were determined by logistic regression analysis. Predictors of MPCs declining in CHF subjects were examined in stepwise logistic regression. *C*-statistics, integrated discrimination indices (IDI), and net-reclassification improvement (NRI) were utilized for prediction performance analyses. Odds ratio (OR) and 95% confidence interval (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

Study patient population

Our study consisted of 136 patients with CAD who were admitted to the hospital with ADHF and had stable severe chronic cardiac failure (NYHA-III/IV) at discharge. All the subjects were divided into two cohorts depending on whether a fatal event occurred within the 3-year post-discharge observation period. Median follow-up was 2.12 years. Twenty-three participants died and CHF-related death occurred in 18 (78.3%) patients. Three subjects (13.0%) died suddenly and two patients (8.7%) died due to myocardial infarction. No other causes of death were defined. Additionally, 86 subjects were hospitalized repetitively due to worsening CHF [17 cases in the dead cohort (73.9%) and 68 cases (66.0%) in the survivors cohort]. Patient demographic characteristics are summarized in Table 1.

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), GFR, glycated hemoglobin (HbA1c), fasting blood glucose level, blood creatinine level, TC, LDL-C, HDL-C, hs-CRP, and number of coronary vessels damaged. No difference was found between the two cohorts in systemic office systolic and diastolic blood pressure (BP) and heart rate (HR). Documented incidence of type 2 diabetes mellitus in patients of the two cohorts was 34.9% and 39.8% ($p = 0.28$), respectively. Proportions of the patients in both cohorts with other cardiovascular risk factors, such as hypertension and dyslipidemia, were similar. Note that there appeared no statistically significant change in global LVEF or diastolic performances (E/Am and E/Em) between the two cohorts. At the same time, the level of circulating NT-pro-BNP was statistically significantly higher in patients who died than in those who survived. Increased NT-pro-BNP > 30% in circulation within hospitalization period was found in 65.2% and 59.2% in both patient cohorts ($p = 0.042$). When analyzing details of pharmacotherapy, no substantial differences apart from levosimendan and antiplatelets were found between the two cohorts with regard to administration of the majority of drugs. Inotropic support with levosimendan use was provided in 13.0% and 6.7% cases in both patient cohorts ($p = 0.026$).

Circulating EMPs and MPCs level in survivors and dead patients

Medians of circulating levels of EMPs in both patient cohorts (survivors and dead subjects) were 0.286 n/mL (95% CI = 0.271–0.309 n/mL) and 0.673 n/mL (95% CI = 0.65–0.74 n/mL) ($p < 0.001$). The data shown that EMPs number in plasma was directly related to NYHA class of CHF ($r = 0.514$, $p = 0.001$), NT-pro-BNP ($r = 0.416$,

Table 1
General characteristic of patient population at discharge from the hospital.

Variable	Died subjects $n = 23$	Survived subjects $n = 103$	p-value
Age, years	56.90 ± 6.33	58.10 ± 7.15	0.66
Males, *n* (%)	13 (56.5%)	49 (47.6%)	0.42
Hypertension, *n* (%)	12 (52.2%)	51 (45.5%)	0.47
Dyslipidemia, *n* (%)	10 (43.5%)	47 (45.6%)	0.62
T2DM, *n* (%)	8 (34.9%)	41 (39.8%)	0.28
Smoking, *n* (%)	7 (30.4%)	27 (26.2%)	0.22
NYHA III class, *n* (%)	17 (73.9%)	73 (70.9%)	0.54
NYHA IV class, *n* (%)	6 (26.1%)	30 (29.1%)	0.56
BMI, kg/m^2	23.2 (95% CI = 22.2–26.9)	24.5 (95% CI = 21.3–27.2)	0.52
eGFR, mL/min/1.73 m^2	90.5 (95% CI = 79.9–108.3)	94.7 (95% CI = 75.1–110.3)	0.61
HbA1c, %	6.5 (95% CI = 4.3–8.5)	6.9 (95% CI = 4.2–8.9)	0.66
Fasting blood glucose, mmol/L	4.64 (95% CI = 3.9–8.1)	5.20 (95% CI = 3.8–9.0)	0.72
Creatinine, μmol/L	92.5 (95% CI = 79.1–113.1)	89.2 (95% CI = 78.5–110.5)	0.56
TC, mmol/L	5.3 (95% CI = 4.6–6.0)	5.0 (95% CI = 4.2–5.8)	0.48
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)	0.48
hs-CRP, mg/L	7.31 (95% CI = 4.60–11.72)	6.97 (95% CI = 4.15–10.85)	0.52
NT-pro-BNP, pg/mL	2237.1 (95% CI 1044.5–3590.1)	1536.3 (95% CI 1004.8–2170.5)	0.001
Increased NT-pro-BNP > 30% within 24–48 h of hospitalization period, *n* (%)	15 (65.2%)	61 (59.2%)	0.042
EMPs, n/mL	0.673 (95% CI = 0.65–0.74)	0.286 (95% CI = 0.271–0.309)	0.001
MPCs, n/μL	0.12 (95% CI = 0.098–0.14)	0.28 (95% CI = 0.26–0.30)	0.001
EMPs to MPCs ratio	78.9 (95% CI = 53.0–116.6)	8.4 (95% CI = 7.6–9.2)	0.001
Systolic BP, mmHg	124 ± 5	128 ± 5	0.52
Diastolic BP, mmHg	72 ± 4	75 ± 5	0.56
Heart rate, beats per 1 min	78 ± 5	76 ± 4	0.44
LVEF, %	42.80 ± 0.76	46.10 ± 0.90	0.026
E/Am, U	16.6 ± 0.94	16.5 ± 1.20	0.44
E/Em, U	16.6 ± 1.00	16.6 ± 0.84	0.46
One-vessel coronary arteries lesion, *n* (%)	5 (21.7%)	24 (23.3%)	0.72
Two-vessel coronary arteries lesion, *n* (%)	8 (34.8%)	40 (38.8%)	0.75
Multivessel lesion, *n* (%)	10 (43.4%)	39 (37.9%)	0.68
Vasodilators i/v, *n* (%)	23 (100%)	100 (97.1%)	0.82
Levosimendan, *n* (%)	3 (13.0%)	7 (6.7%)	0.026
Loop diuretics, *n* (%)	23 (100%)	103 (100%)	0.88
ACEI or ARA, *n* (%)	23 (100%)	103 (100%)	0.88
Mineralocorticoid receptor antagonists, *n* (%)	15 (65.2%)	65 (63.1%)	0.76
Acetylsalicylic acid, *n* (%)	19 (82.6%)	97 (94.2%)	0.044
Other antiplatelets, *n* (%)	4 (17.4%)	6 (5.8%)	0.048
Statins, *n* (%)	10 (43.5%)	47 (45.6%)	0.62
Metformin, *n* (%)	8 (34.9%)	41 (39.8%)	0.28

Note: CI, confidence interval; CAD, coronary artery disease; T2DM, type two diabetes mellitus; TC, total cholesterol; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; 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; ARA, angiotensin-2 receptor antagonist; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; EMPs, endothelial-derived apoptotic microparticles; MPCs, mononuclear progenitor cells.

$p = 0.001$), increased NT-pro-BNP > 30% within 24–48 h of admission period ($r = 0.404$, $p = 0.001$), hs-CRP ($r = 0.402$, $p = 0.002$), type 2 diabetes mellitus ($r = 0.398$, $p = 0.003$), 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 males), TC ($r = 0.313$, $p = 0.001$), and inversely to LVEF ($r = -0.496$, $p = 0.001$) and estimated GFR ($r = -0.408$, $p = 0.003$).

Medians of circulating levels of MPCs in both patient cohorts were 0.28 n/μL (95% CI = 0.26–0.30 n/μL) for survivors and 0.12 n/μL (95% CI = 0.098–0.14 n/μL) for subjects who died ($p = 0.001$). There was a positive association of MPC count with LVEF ($r = 0.639$; $p = 0.001$), E/Em ratio ($r = 0.52$; $p = 0.001$), estimated GFR value ($r = 0.486$; $p = 0.002$); and a negative association with NYHA class of CHF ($r = -0.657$; $p = 0.001$), with type 2 diabetes mellitus ($r = -0.610$; $p = 0.001$), increased NT-pro-BNP > 30% within 24–48 h of admission period ($r = -0.51$, $p = 0.001$), NT-pro-BNP level ($r = -0.473$; $p = 0.001$), creatinine ($r = -0.394$; $p = 0.001$), hs-CRP ($r = -0.356$, $p = 0.003$), LDL cholesterol ($r = -0.354$; $p = 0.001$), and TC level ($r = -0.258$; $p = 0.043$).

Calculated EMPs to MPCs ratios in survivor and dead patient cohorts were 8.4 (95% CI = 7.6–9.2) and 78.9 (95% CI = 53.0–116.6), respectively ($p = 0.001$). EMPs to MPCs ratio were directly related to NYHA class of CHF ($r = 0.62$, $p = 0.001$), increased NT-pro-BNP > 30% within 24–48 h of admission period ($r = 0.54$, $p = 0.002$), NT-pro-BNP ($r = 0.513$, $p = 0.001$), hs-CRP ($r = 0.411$, $p = 0.003$), type 2 diabetes mellitus ($r = 0.398$, $p = 0.001$), multi-vessel lesion of coronary arteries ($r = 0.392$, $p = 0.001$), E/Am ($r = 0.387$, $p = 0.002$), E/Em ($r = 0.356$, $p = 0.002$), gender ($r = 0.396$, $p < 0.001$ for male), TC ($r = 0.322$, $p = 0.001$), age ($r = 0.301$, $p = 0.001$), smoking ($r = 0.287$, $p = 0.001$), and inversely to LVEF ($r = -0.506$, $p = 0.001$) and estimated GFR value ($r = -0.502$, $p = 0.001$). No significant association between the EMPs to MPCs ratio with fasting plasma glucose, HbA1c, mean systolic and diastolic blood pressure, and medications for both cohorts of patients was found.

For further analysis the values of EMPs to MPCs ratio for the entire patient population included in the study obtained by calculation were distributed by quartiles (Q): Q1 (median = 9.7;

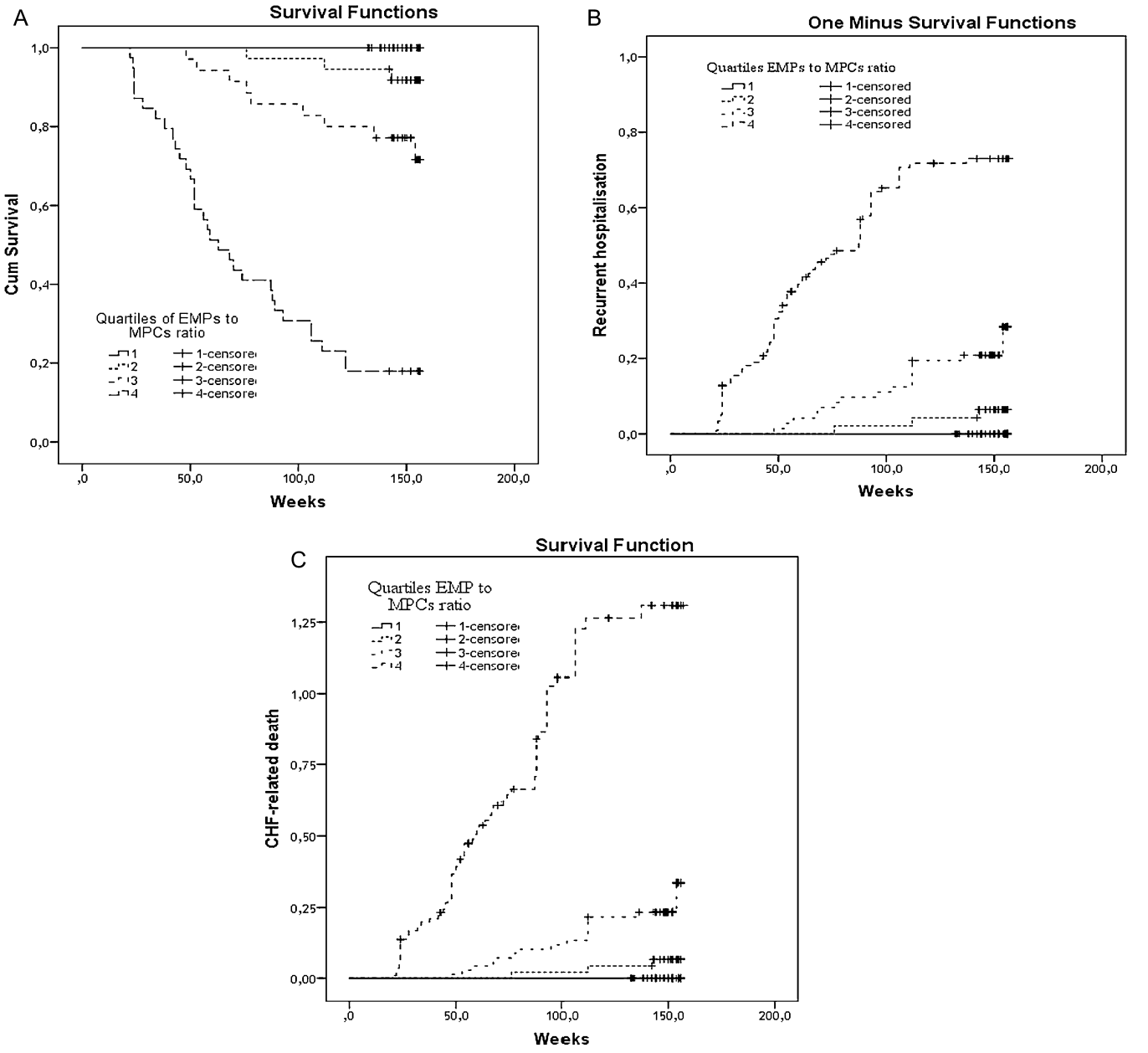


Fig. 1. Results of Kaplan–Meier survival analysis in four cohorts' patients dependent on quartiles of EMPs to MPCs ratio. (A) The cumulative survival; (B) Recurrent hospitalization; (C) CHF-related death. *Note*: Each quartile is enumerated as follows: 1 – quartile I; 2 – quartile II; 3 – quartile III, and 4 – quartile IV. Censored data are marked on the curves suitable for appropriate quartile. EMP, endothelial-derived apoptotic microparticles; MPCs, mononuclear progenitor cells; CHF, chronic heart failure.

95% CI = 5.8–10.6); Q2 (median = 18.2; 95% CI = 11.0–22.2); Q3 (median = 41.4; 95% CI = 22.5–57.8); and Q4 (median = 73.0; 95% CI = 58.9–96.6).

The predictive value of EMPs to MPCs ratio in study patient population

Using Kaplan–Meier survival analysis we found a significantly divergence of survival curves in patients with top quartile (Q4) of EMPs to MPCs ratio when compared with low quartiles (Q1–Q3). The curves' divergence of events accumulation reached a statistical significance in 50 weeks of observation period ($p < 0.001$ for all cases). No statistically significant differences between survival in patient cohorts with Q1 and Q2, as well as Q2 and Q3 EMPs to MPCs ratio were found. The divergence between the two patient cohorts with Q1 and Q3 EMPs to MPCs ratio reached significance at the end of the study. These findings were similar for cumulative survival (Fig. 1A), for recurrent hospitalization (Fig. 1B), and for CHF-related death (Fig. 1C).

Multivariate logistic regression was used to assess whether any combination of assays was able to better discriminate between surviving and dead patients. In the logistic regression analysis, the main factors independently related with cumulative mortality and CHF-related rehospitalization were EPMs, MPCs, EPMs to MPCs ratio, NT-pro-BNP, increased NT-pro-BNP > 30% within 24–48 h of admission period, NYHA class of CHF at discharge, and LVEF. However, EPMs to MPCs ratio had independent predictive potential for all-cause mortality (OR = 1.62; 95% CI = 1.44–1.88; $p = 0.001$), CHF-related death (OR = 1.34; 95% CI = 1.22–1.50; $p = 0.002$), and also CHF-related rehospitalization (OR = 1.30; 95% CI = 1.12–1.52; $p = 0.001$) when compared with other variables (Table 2).

Table 2
Independent variables for three-year all-cause mortality, CHF-related death, and CHF-related rehospitalization.

Variables	All-cause mortality			CHF-related death			CHF-related re-hospitalization		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
EMPs at discharge	1.58	1.20–1.88	0.001	1.22	1.12–1.36	0.001	1.20	1.11–1.32	0.001
MPCs at discharge	1.21	1.06–1.43	0.002	1.15	1.03–1.28	0.001	1.06	1.02–1.09	0.001
EMPs/MPCs ratio at discharge	1.62	1.44–1.88	0.001	1.34	1.22–1.50	0.002	1.30	1.12–1.52	0.001
NYHA class of CHF at discharge	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 at discharge	1.09	1.02–1.16	0.002	1.42	1.22–1.73	0.006	1.44	1.28–1.67	0.002
Increased NT-pro-BNP > 30% within admission period	1.12	1.06–1.18	0.003	1.18	1.03–2.40	0.002	1.16	1.10–1.22	0.002
LVEF	1.08	0.92–1.13	0.048	1.06	0.98–1.11	0.044	1.07	1.02–1.11	0.032
hs-CRP	1.06	1.02–1.09	0.012	1.10	0.92–1.26	0.003	1.04	0.88–1.16	0.002

CHF, chronic heart failure; OR, odds ratio; CI, confidence interval; EMPs, endothelial-derived apoptotic microparticles; MPCs, mononuclear progenitor cells; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein.

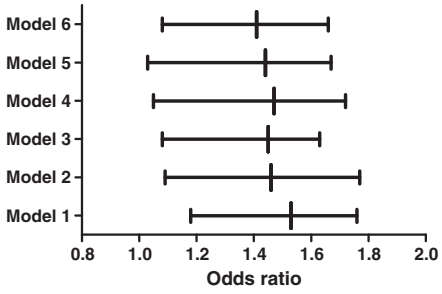
Variables of MPCs, EMPs, NYHA class, NT-pro-BNP, and increased NT-pro-BNP > 30% within admission period remained statistically significant for all categories of clinical events: all-cause mortality, CHF-related death, and CHF-related rehospitalization, whereas LVEF and hs-CRP for all variables did not. Using a stepwise model selection method for multivariable prediction model we investigated the summary effect of any combinations of EPMs to MPCs ratio, NT-pro-BNP, increased NT-pro-BNP > 30% within 24–48 h of admission period on all-cause mortality, CHF-related death, and CHF-related rehospitalization (Table 3). A stepwise model selection method demonstrated that NT-pro-BNP at discharge, increased NT-proBNP > 30% within 24–48 h of admission period, NYHA class of CHF at discharge, LVEF, hs-CRP added to EPMs to MPCs ratio do not offer additional discriminatory potential.

Using reclassification methods, however, we found that the addition of EPMs to MPCs ratio to the ABC model (NT-pro-BNP, increased NT-pro-BNP > 30%) improved the relative IDI by 19.6% for all-cause mortality, by 21.7% for CHF-related death, and by 19.5% for CHF-related rehospitalization (Table 4). For category-free NRI, 14% of events ($p = 0.001$) and 23% of non-events ($p = 0.0001$) were correctly reclassified by the addition of EPMs to MPCs ratio to the ABC model for all-cause mortality (Table 5). When EPMs to MPCs ratio was added to the ABC model, 15% of events ($p = 0.001$) and 29% of non-events ($p = 0.002$) were reclassified for CHF-related death. Therefore, 13% of events ($p = 0.001$) and 22% of non-events ($p = 0.002$) were reclassified for CHF-related rehospitalization. Thus, we suggest that EPMs to MPCs ratio remained a statistically significant predictor for combined end-point including all-cause mortality and CHF-related readmission.

Discussion

Well known that levels of circulating microparticles are enhanced in a large number of pathological states including cardiovascular diseases, such as CHF and metabolic disorders, and this has been linked to deleterious effects on cells from the vascular wall, mainly, endothelial cells. Circulating microparticles have been considered as biomarkers as well as potential mediators of biological messages between different type cells. Endothelial microparticles are complex vesicular structures that originate from plasma membranes of activated or apoptotic endothelial cells [31]. While endothelial-derived apoptotic microparticles could compromise vascular homeostasis and then represent key players in the pathogenesis of several inflammatory and thrombotic diseases, their role in cardiovascular diseases is not understood. Circulating EMPs are increased in CAD, hypertension, atrial fibrillation, peripheral vascular disease, and also CHF [32–34]. However, they

Table 3
Predictive value of EPMs to MPCs ratio for combined end-point (all-cause mortality and CHF-related rehospitalization). Multivariable prediction model shows lack of additional information to discriminate between survivors and dead patients with ischemic severe CHF when NT-proBNP, NYHA class of CHF at discharge, LVEF, and hs-CRP were added to predicted model based on EPMs to MPCs ratio.



Prognostic model	OR	95% CI	*p*-value
Model 6	1.41	1.08–1.66	0.001
Model 5	1.44	1.03–1.67	0.002
Model 4	1.47	1.05–1.72	0.001
Model 3	1.45	1.08–1.63	0.003
Model 2	1.46	1.09–1.77	0.001
Model 1	1.53	1.18–1.76	0.001

Notes: Model 1: EPMs to MPCs ratio; Model 2: EPMs to MPCs ratio + NT-pro-BNP at discharge; Model 3: EPMs to MPCs ratio + NT-pro-BNP + increased NT-pro-BNP > 30% within admission period; Model 4: EPMs to MPCs ratio + NT-pro-BNP + increased NT-pro-BNP > 30% within admission period + NYHA class of CHF at discharge; Model 5: EPMs to MPCs ratio + NT-pro-BNP + increased NT-pro-BNP > 30% within admission period + NYHA class of CHF at discharge + LVEF; Model 6: EPMs to MPCs ratio + NT-pro-BNP + increased NT-pro-BNP > 30% within admission period + NYHA class of CHF at discharge + LVEF + hs-CRP.
EMPs, endothelial-derived apoptotic microparticles; MPCs, mononuclear progenitor cells; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein.

Table 4
C-statistics for models with EPMs to MPCs ratio, NT-pro-BNP, increased NT-pro-BNP > 30% within 24–48 h of admission period as continuous variables.

Models	AUC (95% CI)	ΔAUC	IDI (±SE)	Relative IDI (%)
All-cause mortality				
Model 1 (NT-pro-BNP, increased NT-pro-BNP > 30%)	0.683	–	–	–
Model 1 + EPMs to MPCs ratio	0.834	–	–	–
Model 1 + EPMs to MPCs ratio vs Model 1	–	0.151; $p = 0.001$	0.03 ± 0.011	19.6%
CHF-related death				
Model 1 (NT-pro-BNP, increased NT-pro-BNP > 30%)	0.646	–	–	–
Model 1 + EPMs to MPCs ratio	0.872	–	–	–
Model 1 + EPMs to MPCs ratio vs Model 1	–	0.226; $p = 0.0001$	0.07 ± 0.015	21.7%
CHF-related rehospitalization				
Model 1 (NT-pro-BNP, increased NT-pro-BNP > 30%)	0.657	–	–	–
Model 1 + EPMs to MPCs ratio	0.854	–	–	–
Model 1 + EPMs to MPCs ratio vs Model 1	–	0.197; $p = 0.0012$	0.06 ± 0.012	19.5%

Note: Relative IDI – calculated as the ratio of IDI over the discrimination slope of the model without EPMs to MPCs ratio.
AUC, area under the curve; SE, standard error; IDI, integrated discrimination index; MPCs, mononuclear progenitor cells; EMPs, endothelial-derived apoptotic microparticles; NT-pro-BNP, N-terminal pro-brain natriuretic peptide.

are considered as a biomarker of vascular damage and are proposed to be a potent marker of ischemic risk. Sinning et al. [35] reported that the level of circulating EMPs with apoptotic phenotype CD31+/Annexin V+ is an independent predictor of cardiovascular events in stable CAD patients and may be useful for risk stratification. Moreover, EMPs may contribute inflammatory, thrombogenic, and proteolytic pathways that are suitable for acute and acutely decompensated CHF [19]. The main advantage of this biomarker is the lack of tight interrelationship between numerous of EMPs in blood and creatinine clearance, GFR, and sex that is a notably important issue in acutely decompensated CHF patients. Mononuclear progenitor cells are mobilized into circulation to replace damaged endothelium and recapitulate the vascular network of injured tissues. Numerous studies have reported that MPCs with proangiogenic phenotype CD14+CD309(VEGFR2)+Tie-2+ play an important role in vascular repair and restoration of endothelial function [19,36,37]. Therefore, significant variability in the degree of change in EMPs and MPCs levels between individuals with ischemic CHF raises the interesting possibility that they could be a predictive biomarker in this patient population [38]. We previously reported that a decreased circulating level of CD14+CD309+Tei2+ MPCs is related to a number of cardiovascular risk factors in patients with known CAD and CHF [17,39]. Alba et al. [37] found that a decrease in CD34+VEGFR2+ cells was independently associated with functional capacity of CHF patients and increased mortality. However, predictive potential of both CD31+/Annexin V+ EMPs and CD14+CD309(VEGFR2)+Tie-2+ MPCs when compared with brain natriuretic peptides and hs-CRP is still not clear.

Table 5
Prediction performance analyses for models with EPMs to MPCs ratio, NT-pro-BNP, increased NT-pro-BNP > 30% within 24–48 h of admission period as continuous variables.

Model 2 vs model 1	
All-cause mortality	
Categorical NRI	0.13 (95% CI 0.09–0.17)
Percentage of events correctly reclassified	7 ($p = 0.12$)
Percentage of non-events correctly reclassified	9 ($p = 0.023$)
Categorical free NRI	0.57 (95% CI 0.44–0.69)
Percentage of events correctly reclassified	14% ($p = 0.001$)
Percentage of non-events correctly reclassified	23% ($p = 0.0001$)
CHF-related death	
Categorical NRI	0.15 (95% CI 0.12–0.17)
Percentage of events correctly reclassified	4 ($p = 0.11$)
Percentage of non-events correctly reclassified	7 ($p = 0.012$)
Categorical free NRI	0.42 (95% CI 0.31–0.57)
Percentage of events correctly reclassified	15% ($p = 0.001$)
Percentage of non-events correctly reclassified	29% ($p = 0.002$)
CHF-related rehospitalization	
Categorical NRI	0.11 (95% CI 0.06–0.19)
Percentage of events correctly reclassified	5 ($p = 0.16$)
Percentage of non-events correctly reclassified	5 ($p = 0.018$)
Categorical free NRI	0.37 (95% CI 0.30–0.43)
Percentage of events correctly reclassified	13% ($p = 0.001$)
Percentage of non-events correctly reclassified	22% ($p = 0.001$)

Note: Model 1 – NT-pro-BNP, increased NT-pro-BNP > 30%; Model 2 – NT-pro-BNP, increased NT-pro-BNP > 30% and EPMs to MPCs ratio.
NRI, net reclassification improvement; MPCs, mononuclear progenitor cells; EMPs, endothelial-derived apoptotic microparticles; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; CHF, chronic heart failure.

It has been postulated that imbalance between exaggerated production of EMPs and deficiency of MPCs with proangiogenic potency in circulation may characterize an insufficiency in tissue repair response more closely than each biomarker alone. Moreover, using EMP to MPC ratio may also lead to minimize bias related to age-dependent disease, metabolic disorders, and demographic particularities. We suggest that CD31+/Annexin V+ EMPs to CD14+CD309(VEGFR2)+Tie-2+ MPCs ratio may reflect an imbalance between vascular repair and endothelial dysfunction that is suitable for worsening CHF. Probably, predictive value of EMPs to MPCs ratio will not be worse than circulating NT-proBNP, hs-CRP, and serial measurements of NT-proBNP plasma level in subjects with worsening CHF or ADHF. Indeed, we found that EPMs to MPCs ratio had an independent prognostic potential for all-cause mortality, CHF-related death, and also CHF-related rehospitalization and that calculated predictive value was superior when compared with EPMs at discharge alone, MPCs at discharge alone, NT-pro-BNP at discharge, increased NT-pro-BNP > 30% within 24–48 h of admission period, NYHA class of CHF at discharge, or LVEF at discharge. In multivariable prediction model the summary effect of any combinations of EPMs to MPCs ratio with other variables (NT-pro-BNP, increased NT-pro-BNP > 30% within 24–48 h of admission period) on combined end-point point (all-cause mortality and CHF-related rehospitalization) was not superior to EPMs to MPCs ratio alone. Recent data support the hypothesis that EMPs and MPCs contribute to vascular homoeostasis and the pathogenesis of CHF, including mechanisms of worsening endothelial dysfunction [40,41]. We agree with several investigators in terms that the disproportion in circulating levels of both EMPs and MPCs may trigger differential consequences on cardiovascular remodeling [35,40,41]. Although results of recent studies dedicated to investigation of EPCs and MPCs in CHF were inconsistent and suggested that levels of both biomarkers may vary according to factors such as disease severity, underlying cause of cardiomyopathy and medical therapy, we believe that EPCs to MPCs ratio use as a prognostic marker may diminish this controversy [42]. New

studies with more size and statistical power and clinical follow-up are required to determine the prognostic significance of these findings.

Study limitations

This study has some limitations. The major limitation of this study was the small population size. However, this was not a randomized and controlled study. The authors suggested that a greater cohort of patients with more incidences detected is desirable to improve the credibility of the study. 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 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. 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 and PMCs is also possible and has not been characterized in depth in CHF patients. However, the authors suppose that these restrictions might have no significant impact on the study data interpretation.

Conclusion

We demonstrated that EMPs to MPCs ratio is considered a more important indicator of an imbalance between angiogenic and apoptotic responses with possible relation to cardiovascular outcomes in post-discharge patients with clinical stabilization after ischemic ADHF. The fourth quartile of EMPs to MPCs ratio shows a close association with increased three-year CHF-related death, all-cause mortality, and risk for recurrent admission due to worsening CHF after discharge from the hospital.

Conflict of interest

None declared.

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 State Medical University, Zaporozhye, Ukraine. The study was carried out in conformity with the Declaration of Helsinki.

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