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HEALTHY AGING RESEARCH

Impact of age on pattern of circulating endothelial-derived microparticles in heart failure patients

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Abstract

***Background*:** The objective of the study was to compare apoptotic and activated endothelial-derived microparticle (EMP) patterns in elderly chronic heart failure (CHF) patients.

***Methods*:** Three hundred eighty-eight CHF patients were divided into elderly (n=105) and control cohorts (n=283). Multispiral computed tomography angiography and/or angiograph verified the ischemic nature of the disease. Endothelial-derived apoptotic (Annexin V+) and activated (CD62E+) microparticles were characterized by flow cytometry at baseline.

Results: CD31+/Annexin V+ to CD62E+ ratio was significantly correlated with age (r=0.623; P=0.001), high sensitivity-C reactive protein [hs-CRP] (r = 0.36, P = 0.001), NT-pro-brain natriuretic peptide [BNP] (r = 0.38, P = 0.001), and type II diabetes mellitus [T2DM] (r = 0.36, P = 0.001), and inversely with left ventricular ejection fraction [LVEF] (r = -0.36, P = 0.001). Age, New York Heart Association (NYHA) class, T2DM, LVEF < 45%, serum uric acid, NT-pro-BNP, and hs-CRP were associated with increased CD31+/annexin V+ to CD62E+ ratio by univariate logistic regression. Age of >65 years, LVEF < 45%, NT-pro-BNP, hs-CRP remained statistically significant predictors for increased CD31+/Annexin V+ to CD62E+ ratio by multivariate analysis. Adding age >65 years to the sABC model may improve the relative incremental deterioration index (IDI) for increased CD31+/Annexin V+ to CD62E+ ratio by 10.5%. For category-free net reclassification improvement (NRI), 4% of events (p=0.001) and 7% of non-events (p=0.001) were correctly reclassified by this addition of age > 65 years.

***Conclusions*:** Elderly CHF patients have an increased apoptotic immune phenotype of circulating EMPs and a deficiency of potential angiogenic-activated EMPs.

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Introduction

Chronic heart failure (CHF) is a frequently detected age-related vascular disease and remains a leading cause of cardiovascular morbidity and mortality worldwide [1]. The development of CHF originating with ischemic and non-ischemic causes closely associates with endothelial dysfunction, which appears during the early stages of cardiac failure and mediates tissue injury via microcirculatory function disorders [2-4]. Existing cardiovascular risk factors, metabolic diseases, and comorbidities accelerate endothelial dysfunction in aging and worsen CHF through various molecular mechanisms [5-7]. Direct

endothelial cell injury, free radical overproduction and oxidative state, low intensity inflammation, neurohumoral hyperactivity that are contributory to CHF are recognized as the main causes of endothelial dysfunction [8-10]. Taking together, it has been suggested that biological markers of endothelial cell injury are attractive indicators of the nature evolution of CHF [11]. In this context, endothelial-derived microparticles (EMPs) originated from the plasma membranes of activated or apoptotic endothelial cells may act as novel biomarkers of CHF [12,13]. Although elevated EMPs are found in several diseases including CHF, hypertension, atherosclerosis, myocardial infarction, stroke, diabetes mellitus, autoimmune and rheumatic diseases [14-16], recent evidence regarding their predictive value are controversial especially in elderly patient populations [11,17]. However, the role of EMPs can be thought of in two different ways: i) through activation of endothelial cells; and ii) apoptosis of endothelial cells in elderly CHF patients, which has not been recognized as a direct molecular mechanism of age-related diseases. The objective of this study was to compare apoptotic and activated EMP patterns in elderly CHF patients.

Methods

Study population

The study population consisted of 388 consecutive patients with CHF who underwent angiography or percutaneous coronary intervention (PCI) between April 2010 to June 2014, as well as those who were referred as post-myocardial infarction subjects within this period in our five centers that participated in this investigation. The patients were selected from 1,427 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.

Methods for visualization of coronary arteries

Multispiral computed tomography angiography and/or angiographic study were performed to verify the ischemic nature of the disease in patients. Multispiral computed tomography angiography was performed 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 that the indications for revascularization were available. Coronary artery disease (CAD) was considered for diagnosis upon the availability of previous angiographic examinations carried out within the last six months provided no new cardiovascular events occurred during this period, and the procedure was available for use. The coronary artery wall structure was measured by contrast-enhanced spiral computed tomography angiography [18] on a Somatom Volume Zoom scanner (Siemens, Erlangen, Germany) with two detector rows using non-ionic contrast Omnipaque (Amersham Health, Ireland).

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 a phased transducer of 5 MHz. Left ventricular end-diastolic and end-systolic volumes, and ejection fraction (LVEF) were measured by a modified Simpson's planimetric method [19, 20]. Inter- and intra-observer variability coefficients for LVEF were 3.2% and 1.1% respectively

Glomerular filtration rate measurement

Calculation of glomerular filtration rate (GFR) was calculated by CKD-EPI formula [21].

Biomarker determination

All biomarkers were determined at baseline. To measure of biological marker concentrations, blood samples were drawn in the morning (at 7-8 a.m.) into chilled silicone test tubes. Samples were processed according to the manufacturer's recommendations for the analytical technique used. The samples were centrifuged upon permanent cooling at 6,000 rpm for 3 minutes. Then, plasma was frozen immediately at -70^{o}C until measurement.

Circulating NT-pro-BNP level was measured by immunoelectrochemoluminescent assay using kits provided by Elecsys kits (Roche, Mannheim, GermanyUSA) on an Elecsys 1010 analyzer (Roche, Mannheim, Germany). The high-sensitivity C-reactive protein (hs-CRP) levels were measured by using turbidimetric technique on an AU640 analyzer manufactured by Diagnostic Systems Group (Japan).

Concentrations of total cholesterol (TC) and cholesterol of high-density lipoproteins (HDLP) were measured by enzymatic method. Concentration of cholesterol of low-density lipoproteins (LDL-C) was calculated according to the Friedewald formula (1972) [22].

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

Endothelial-derived apoptotic and activated microparticles determination

Endothelial-derived apoptotic and activated microparticles were phenotyped by flow cytometry using a 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 the high-definition fluorescence-activated cell sorter (HD-FACS) 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 were added. For each sample, 500,000 events were analyzed. The EMP gate was defined by size, using 0.8 and 1.1 μm latex 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 the most specific EMPs [23, 24].

Statistical analysis

Statistical analysis of the results 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-tests or Mann-Whitney U-tests were used. To compare categorical variables between groups, Chi square test ($\chi 2$) and Fisher's F exact test were used. The factors that could be potentially associated with elevated EMPs were determined by log regression analysis. Reclassification methods (C-statistics) were utilized for prediction performance analyses using net reclassification improvement (NRI) and incremental deterioration index (IDI). A calculated difference of $P < 0.05$ was considered significant.

Results

Study patient population

The characteristics of the patients who participated in the study are depicted in Table 1. At baseline, mean age in both 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 II diabetes mellitus and obesity, were reported in 66.0%; 37.6%; and 44.3% of the population, respectively. The mean left ventricular ejection fraction appears to be slightly decreased. Mildly increased hs-CRP and sufficiently raised NT-pro-BNP were found in the entire cohort. Elderly subjects frequently presented III NYHA class, lower estimated glomerular filtration ratio, as well as higher uric acid, creatinine level, NT-pro-BNP, hs-CRP, and lipid abnormalities when compared with control patient cohort.

Concomitant treatment of CHF

The majority patients with CHF were treated with ACE inhibitors or ARBs, beta-adrenoblockers, the I/f blocker ivabradine, mineralocorticoid receptor antagonists (preferable eplerenone), and antiplatelet drugs (Table 2). Adding loop diuretics was done when fluid retention was determined. Dihydropyridine derivate of calcium channel blockers (amlodipine or lacidipine) were prescribed for hypertension treatment. Metformin and / or sitagliptin were used in type II diabetic patients as a component of contemporary treatment of CHF. Elderly patients were treated frequently with loop diuretics and aspirin (by 100% and 86.7% respectively). In opposite, beta-adrenoblockers (by 86.6%) and sitagliptin (by 13.8%) were given frequently among patients in control cohort.

Patterns of circulating endothelial-derived microparticles

As shown in Fig. 1A, total numbers of $CD144^+$/annexin V^+ phenotyped endothelial-derived microparticles (EMPs) were not different between both patient cohorts (P=0.26). A double-positive CD144 and CD31-staining phenotype of EMPs was slightly increased in elderly patients when compared with the control cohort subjects (Fig. 1B). Therefore, Fig. 1C presented that $CD144^+$/$CD31^+$/annexin V^+ EMPs were elevated significantly in elderly subjects when compared with patients who were enrolled in the control cohort (P=0.001). The mean number of circulating apoptotic EMP ($CD31^+$/annexin V^+) in elderly patients was higher when compared with the mean number of EMPs isolated from peripheral blood from whose who were enrolled in the control cohort (P<0.001) (Fig. 1D). In contrast, activated CD62E+ EMP numbers were significantly decreased in the elderly patients when compared with control cohort subjects (P=0.001) (Fig. 1E).

Calculated ratios of the number of apoptotic to activated phenotypes of circulating endothelial-derived microparticles are shown in Table 3. Medians of obtained values were significantly increased in elderly patient cohort when compared with control cohort.

Association between circulating endothelial-derived microparticles and demographic, hemodynamic, other biomarkers

There was a close association between the total numbers of $CD144^+$/annexin V^+ EMPs with NYHA class (r = 0.32, P = 0.002), NT-pro-BNP (r = 0.301, P = 0.001), LVEF (r = -0.26, P = 0.001). 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), 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 labelled as $CD31^+$/annexin V^+ were positively associated with NYHA class (r = 0.53, P = 0.001), NT-pro-BNP (r = 0.49, P = 0.001), age (r = 0.42, P = 0.001), uric acid (r = 0.41, P = 0.001), hs-CRP (0.408, P = 0.006), 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 age (r = -0.502, P = 0.001), 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, and medications for both cohorts of the patients was found.

Therefore, $CD31^+$/annexin V+ to CD62E+ ratio appeared to be the most correlated with age (r=0.623; P=0.001) than other variables. Overall, $CD144^+$/$CD31^+$ to CD62E+ ratio and $CD144^+$/$CD31^+$/annexin V^+ to CD62E+ ratio demonstrated a slight correlation with hs-CRP (r=0.38, P = 0.002 and r=0.45, P = 0.001 respectively), NYHA class (r = 0.23, P = 0.001 and r = 0.29, P = 0.001 respectively), NT-pro-BNP (r = 0.31, P = 0.003 and r = 0.33, P = 0.001 respectively). Conversely, $CD31^+$/annexin V^+ to $CD62E^+$ ratio was significantly correlated with hs-CRP (r = 0.36, P = 0.001), NT-pro-BNP (r = 0.38, P = 0.001), T2DM (r = 0.36, P = 0.001), and inversely with LVEF (r = -0.36, P = 0.001).

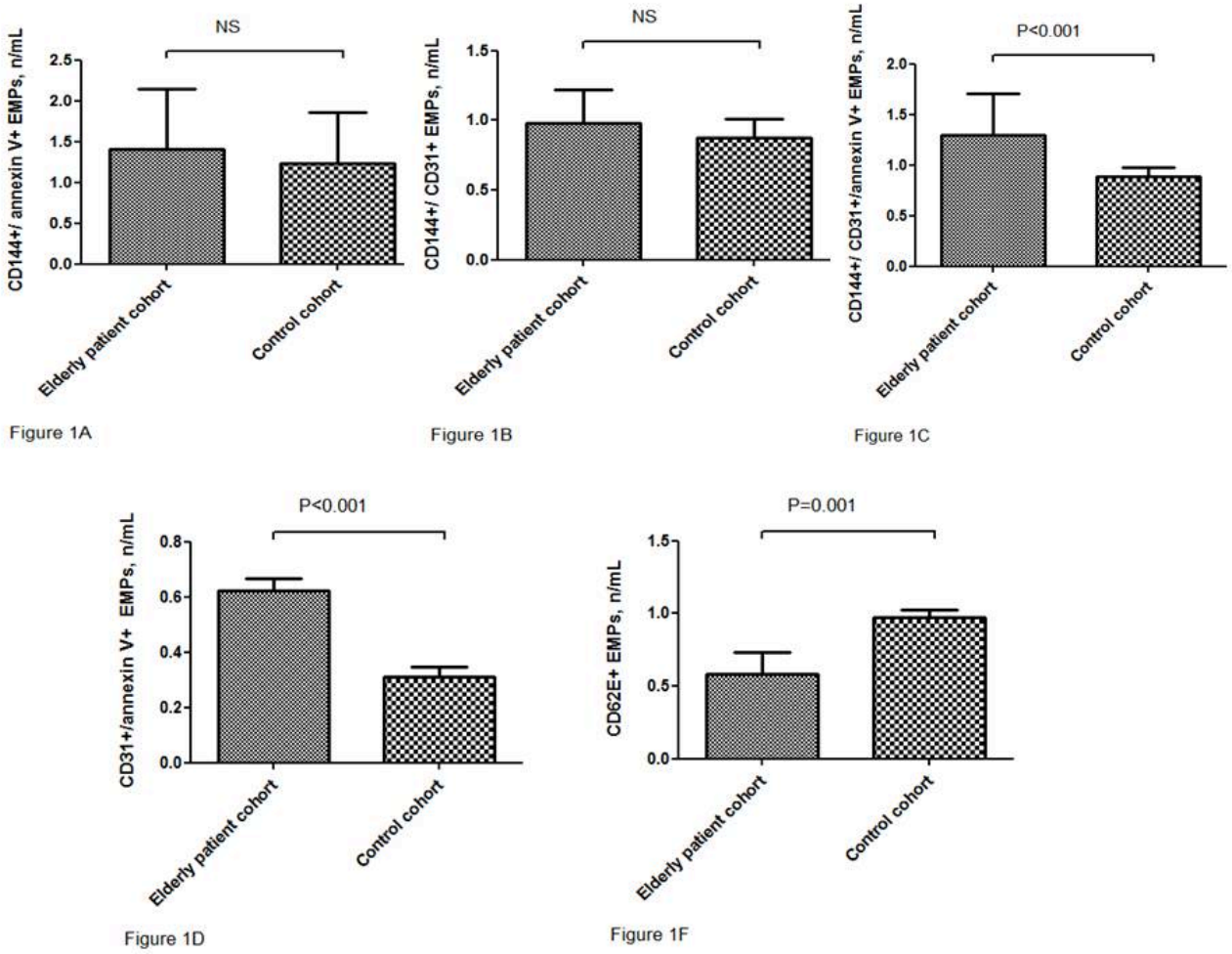

Figure 1. Comparison of the profile of circulating endothelial-derived microparticles in the elderly patient cohort and the control cohort

Values for all figures are mean and standard deviation (SD) and were compared using a Student's paired t-test
Figure 1A shows CD144+/ Annexin V+ EMP numbers isolated from peripheral blood from both cohort patients
Figure 1B shows circulating CD144+/ CD31+ EMPs in both cohort patients
Figure 1C presents CD144+/ CD31+/Annexin V+ EMP numbers isolated from circulation from both cohort patients
Figure 1D shows CD31+/Annexin V+ EMP numbers isolated from peripheral blood from both cohort patients
Figure 1E shows CD62E+EMP numbers isolated from peripheral blood from both cohort patients

Predictors of changes of circulating endothelial-derived microparticle pattern

Univariate and multivariate logistic regression were used to assess whether any factors were able to predict changes of circulating endothelial-derived microparticle pattern assayed using CD31+/Annexin V+ to CD62E+ ratio. Univariate logistic regression analysis shown that the main factors related with increased CD31+/annexin V+ to CD62E+ ratio were age, NYHA class, T2DM, LVEF < 45%, serum uric acid, NT-pro-BNP, and hs-CRP (Table 4). In a multivariate model, age >65 years, LVEF < 45%, NT-pro-BNP, hs-CRP were statistically significant predictors for elevated CD31+/annexin V+ to CD62E+ ratio.

Table 1. Characteristics of the study participants

	Entire patient cohort (n=388)	Elderly patient cohort (n=105)	Control cohort (n=283)	P value
Mean age, years	58.34±9.60	67.12±3.23	53.92±5.12	0.042
Male, n (%)	207 (53.3%)	59 (56.2%)	148 (52.3%)	0.84
I NYHA class, n (%)	77 (19.8%)	22 (21.0%)	55 (19.4%)	0.95
II NYHA class, n (%)	147 (37.9%)	26 (24.7%)	121 (42.7%)	0.001
III NYHA class, n (%)	83 (21.4%)	35 (33.3%)	48 (17.0%)	0.001
IV NYHA class, n (%)	81 (20.9%)	22 (21.0%)	59 (20.9%)	0.96
Hypertension, n (%)	214 (55.5%)	57 (54.3%)	157 (55.6%)	0.98
Dyslipidemia, n (%)	256 (66.0%)	63 (60.0%)	193 (68.2%)	0.12
Type 2 diabetes mellitus, n (%)	146 (37.6%)	37 (35.2%)	109 (38.5%)	0.88
Obesity, n (%)	172 (44.3%)	47 (44.8%)	125 (44.2%)	0.98
Adherence to smoke, n (%)	76 (19.6%)	23 (21.9%)	53 (18.7%)	0.78
BMI, kg/m^2	24.1 (95% CI: 21.6-28.7)	23.5 (95% CI: 20.5-25.7)	23.3 (95% CI: 21.5-24.8)	0.68
Systolic BP, mm Hg	131±8	132±5	133±5	0.84
Diastolic BP, mm Hg	78±5	79±4	78±4	0.92
Heart rate, beat per min.	70.52±3.34	74.50±5.5	68.60±6.8	0.42
LVEF, %	42.80±5.76	42.03±3.15	43.50±6.44	0.76
GFR, 1,73 ml/ min/ m^2	82.3 (95% CI = 68.7 – 102.6)	80.9 (95% CI=70.1–94.6)	83.2 (95% CI=77.5–103.2)	0.042
Creatinine, µmol/L	72.3 (95% CI = 58.7 – 92.6)	79.2 (95% CI=65.5–98.1)	70.1 (95% CI = 59.1 – 88.6)	0.048
Fasting glucose, mmol/L	5.20 (95% CI =3.3-9.7)	5.21(95% CI=3.5-9.2)	4.96 (95% CI=3.5-8.2)	0.32
HbA1c, %	6.8 (95% CI =4.1-9.5)	6.9 (95% CI=4.3-9.4)	6.6 (95% CI=4.6-8.3)	0.36
Hemoglobin, g/L	135.4 (95% CI = 128.5 – 140.1)	133.5 (95% CI = 126.0 – 136.1)	136.1 (95% CI = 125.1 – 144.8)	0.06
Total cholesterol, mmol/L	5.1 (95% CI = 3.9 – 6.1)	5.2 (95% CI=4.5-6.2)	5.0 (95% CI = 3.4 – 5.8)	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)	35.9 (95% CI = 25.1 – 40.8)	31.1 (95% CI = 20.6 – 36.9)	0.036
NT-pro-BNP, pg/mL	1977.2 (95% CI 984.7 – 2993.2)	2776.1 (95% CI 1077.3 – 3952.1)	1530.6 (95% CI = 644.5 – 2560.6)	0.042
hs-CRP, mg/L	7.34 (95% CI =6.77-7.95)	8.02 (95% CI =6.32-9.92)	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 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, HbA1c – glycated hemoglobin, HDL - high-density lipoprotein; LDL - Low-density lipoprotein; hs-CRP – high sensitive C-reactive protein

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

	Entire patient cohort (n=388)	Elderly patient cohort (n=105)	Control cohort (n=283)	P value
ACE inhibitors or ARBs, n (%)	388 (100%)	105 (100%)	283 (100%)	1.0
Aspirin, n (%)	305 (78.6%)	91 (86.7%)	214 (75.6%)	0.026
Other antiplatelet drugs, n (%)	83 (21.4%)	14 (13.3%)	69 (24.4%)	0.001
Beta-adrenoblockers, n (%)	324 (83.5%)	79 (75.2%)	245 (86.6%)	0.024
Dihydropyridine calcium channel blockers, n (%)	63 (16.2%)	17 (16.2%)	46 (16.3%)	0.98
Ivabradine, n (%)	137 (35.3%)	38 (36.2%)	99 (35.0%)	0.82
Mineralocorticoid receptor antagonists, n (%)	152 (39.2%)	40 (38.1%)	112 (39.6%)	0.88
Loop diuretics, n (%)	311 (80.1%)	105 (100%)	206 (72.8%)	0.043
Statins, n (%)	256 (66.0%)	63 (60.0%)	193 (68.2%)	0.82
Metformin, n (%)	146 (37.6%)	37 (35.2%)	109 (38.5%)	0.88
Sitagliptin, n (%)	48 (12.4%)	9 (8.5%)	39 (13.8%)	0.001

Notes: P value was calculated between variables for subjects who experienced the composite endpoint and did not; data were presented as numbers and frequency; ACE – angiotensin-converting enzyme; ARBs – angiotensin-2 receptors blockers

Table 3. Ratios of numbers of apoptotic to activated phenotypes of circulating endothelial-derived microparticles

	Entire patient cohort (n=388)	Elderly patient cohort (n=105)	Control cohort (n=283)	P value for comparison of both cohorts
CD144+/annexin V+ to CD62E+ ratio	3.11 (IQR = 2.14 – 4.15)	2.33 (IQR = 1.77 – 3.05)	3.75 (IQR = 3.15 – 4.43)	0.001
CD144+/CD31+ to CD62E+ ratio	2.54 (IQR = 2.10 – 3.08)	2.03 (IQR = 1.67 – 2.45)	2.86 (IQR = 2.44 – 3.21)	0.001
CD144+/ CD31+/annexin V+ to CD62E+ ratio	2.68 (IQR = 2.15 – 3.22)	2.26 (IQR = 2.09 – 2.48)	3.06 (IQR = 2.67 – 3.53)	0.001
CD31+/annexin V+ to CD62E+ ratio	0.702 (IQR 0.40 = 1.19)	1.15 (IQR = 0.92 – 1.41)	0.301 (IQR = 0.188 – 0.425)	0.001

Note: Data are presented as median and interquartile range (IQR). Activated phenotype defined as $CD62E^{+}$; apoptotic phenotypes are labeled with $CD144^{+}$/ $CD31^{+}$ in combination with annexin V^{+}

Table 4. Univariate and multivariate regression analysis

Variances	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
CD31+/annexin V+ to CD62E+ ratio						
Age (>65 years vs < 65 years)	1.16	1.09-1.22	0.003	1.10	1.05-1.15	0.001
NYHA class	1.05	1.02-1.09	0.002	1.02	0.97-1.06	0.001
T2DM (present vs absent)	1.02	1.01-1.05	0.003	1.02	0.98-1.02	0.044
LVEF less 45% (present vs absent)	1.04	1.01-1.06	0.001	1.03	1.01-1.04	0.003
Uric acid per 10 mmol/L	1.08	1.03-1.09	0.001	1.03	0.92-1.08	<0.05
NT-pro-BNP per 400 pg/mL	1.04	1.02-1.07	0.001	1.02	1.01-1.05	0.001
hs-CRP per 1 mg/L	1.10	1.06-1.13	0.001	1.08	1.03-1.10	0.002

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

We used reclassification methods to define whether the addition of age >65 years to the standard ABC model consisting of LVEF < 45%, NT-pro-BNP, and hs-CRP improved the discriminate value of the model. The results showed that adding age >65 years to the standard ABC model may improve the relative IDI for increased $CD31^{+}$/Annexin V+ to CD62E+ ratio by 10.5%. For category-free NRI, 4% of events (p=0.001) and 7% of non-events (p=0.001) were correctly reclassified by the addition of age > 65 years to the ABC model. Thus, apoptotic phenotype of circulating EMPs in CHF patients may relate to age.

Predictors of changes of circulating endothelial-derived microparticle pattern

Univariate and multivariate logistic regression were used to assess whether any factors were able to predict changes of circulating endothelial-derived microparticle pattern assayed using CD31+/Annexin V+ to CD62E+ ratio. Univariate logistic regression analysis shown that the main factors related with increased CD31+/annexin V+ to CD62E+ ratio were age, NYHA class, T2DM, LVEF < 45%, serum uric acid, NT-pro-BNP, and hs-CRP (Table 4). In a multivariate model, age >65 years, LVEF < 45%, NT-pro-BNP, hs-CRP were statistically significant predictors for elevated CD31+/annexin V+ to CD62E+ ratio.

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Discussion

It is known that age is a powerful risk factor for cardiovascular diseases including cardiac failure. We tested the hypothesis that elderly people with CHF my have different patterns of EMP expression compared with younger persons with CHF. The results of our study have shown that the respective deficiency of the number of activated EMPs in elderly patients with CHF is associated with increased circulating apoptotic EMPs when compared with the control patient cohort. Thus, the apoptotic pattern of circulating EMPs, but not vascular, inflammatory, or neurohumoral biomarkers, may interplay in the development of CHF in elderly.

Recent clinical studies have shown that apoptotic pattern of EMPs may have utility in predicting plaque instability and atherosclerosis in aging populations, independent of established biomarkers, such as NT-proBNP, hs-CRP, and uric acid [25, 26]. However, our data supports the hypothesis that apoptotic patterns of EMPs contribute to CHF development in elderly persons. Although the exact molecular mechanisms regarding the effect of EMPs on targeting cells in aging are unclear, it has been suggested that an imbalance between apoptotic and activated EMPs affects cell-to-cell cross-talk and plays a pivotal role in vascular tone, angiogenesis, and neovascularization [27, 28]. These effects may be explained in various ways. Several mechanisms involved in CHF in the elderly have been defined as causal for increased apoptotic EMP. There is evidence of diminished apoptotic cell clearance in the aging population [29] that leads to increased coagulation, endothelial cell injury, suppression of nitrous oxide bioavailability, and induction of pro-oxidative and pro-inflammatory effects [30-33] that are suitable for CHF. Therefore, a decrease in activated EMPs, which transport active molecules, regulating peptides, miRNAs, hormones, growth factors, etc., appear to be a negative effect upon angiogenesis and vascular repair, as well as integrative mechanisms for vascular aging [34]. Interestingly, activated EMPs are key players in an important process: cellular oxidative stress resistance that links mitochondrial oxidative stress, inflammation, and the development of CHF [35]. Overall, patterns of circulating EMPs could be considered a tool for reclassification of the elderly patients at risk, because lack of appropriate scores with high precision and accuracy sufficiently limit our efforts for predictive grading in the aging population. However, it will be necessary to support this issue with large clinical studies with more statistical power and an increased sample size.

One limitation of our study is 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 must be further examined. Venous citrated blood drawn from the fistula-free arm was always performed. Blood samples were never frozen before measurement of the microparticles. Although HD-FACS methodology is widely used, theoretically overlap between two or more fluorophores might complicate further interpretation of results. The relatively small sample size may limit the significance of the present study, thus a larger randomized study is necessary to establish the role of EMPs in CHF.

Conclusions

Elderly patients with CHF have circulating EMPS that express an increased apoptotic immune phenotype associated with a deficiency in potential angiogenic-activated EMPs.

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