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Inflammatory phenotype of circulating endothelial-derived microparticles in chronic heart failure patients with metabolic syndrome

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ABSTRACT

Background/Aim: Metabolic syndrome (MetS) may have an adverse impact on cardiovascular events in unselected populations. However, the role of MetS in chronic heart failure (CHF) subjects remains controversial. Endothelial-derived microparticles (EMPs) may play a pivotal role in cell-to-cell cooperation, effects negatively on tissue reparation, and mediates vascular function. Pattern of circulating EMPs probably reflects imbalance between endothelial cell injury and endothelial repair. Aim of the study is to investigate an inflammatory pattern of circulating EMPs in MetS patients with CHF. **Methods:** The study retrospectively evolved 101 patients with MetS (54 subjects with CHF and 47 patients without CHF) without documented coronary artery stenosis >50% at least of one artery and 35 healthy volunteers. Biomarkers were measured at baseline of the study. Circulating EMPs were phenotyped by flow cytometry technique. **Results:** We found CD62E+EMPs and CD62E+ to CD31+/annexin V+ ratio were elevated in healthy persons when compared with MetS patients. CD62E+ to CD31+/annexin V+ ratio was found to be higher in the MetS patients without CHF compared with MetS patients with CHF. Using multiple linear regression analysis, independent impact of based model (body mass index [BMI]), N-terminal brain natriuretic peptide (NT-proBNP), osteoprotegerin (OPG), and high-sensitive C-reactive protein (hs-CRP) on decreased CD62E+ to CD31+/annexin V+ ratio was found. We found that adding of combination of inflammatory biomarkers (hs-CRP, OPG, and NT-proBNP) to the BMI improved the relative integrated discrimination indices by 11.4% for decreased CD62E+ to CD31+/annexin V+ ratio. **Conclusion:** We found that biomarkers of biomechanical stress (NT-proBNP) and inflammation (hs-CRP, OPG) remain statistically significant predictors for decreased CD62E+ to CD31+/annexin V+ ratio in MetS patients with CHF.

KEY WORDS: Cardiovascular risk factors, chronic heart failure, circulating endothelial-derived microparticles, metabolic syndrome

INTRODUCTION

Current clinical, experimental, and epidemiological observations define metabolic syndrome (MetS) as a risk factor clustering related to the development of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [1]. According currently statement, the MetS includes abdominal obesity, insulin resistance (IR), dyslipidemia, elevated blood pressure (BP), and associated with other comorbidities including the prothrombotic and proinflammatory states [2]. Recent studies have emerged that genetic, early-life-depended, age-related, and sociodemographic factors, as well as dietary particularities, coexisting comorbidities are discussed leading causes for the current prevalence of MetS in the general population [3-5]. Therefore, MetS corresponds with several cardiovascular risk factors and associates with an increased incidence of T2DM, cardiovascular events, and mortality [6]. The underlying pathophysiological mechanisms resulting in the MetS, IR, central obesity, are not fully understood. However, activated immunity and cytokine production, oxidative stress may effect on a development of cardiovascular complications through inducing endothelial dysfunction [7-10]. There is evidence that systemic pro-inflammatory response induced by MetS is cause of microvascular endothelial cell inflammation [11,12]. Although the adverse prognostic impact of T2DM in unselected populations and in patients with chronic heart failure (CHF) has been previously shown [13-15], the role of MetS in CHF subjects remains controversial [16-18]. It has suggested that endothelial-derived microparticles (EMPs) may play a pivotal role in cell-to-cell cooperation, negatively affects tissue reparation, and mediates vascular function [19].

Extracellular microparticles are microvesicles with sizes ranging between 50 and 1000 nm released from plasma membrane of wide variety of cells, including endothelial cells, by specific (cytokine stimulation, apoptotic agents, mononuclear cooperation, coagulation, etc.) and non-specific (shear stress) stimuli [20]. Circulating EMPs depending on their origin (apoptotic-derived or activated endothelial cell production) are capable of transferring biological information (regulating peptides, hormones) or even genetic material (micro-RNA, mRNA, and DNA), as well as proteins, lipid components, from one cell to another without direct cell-to-cell contact to maintain cell homeostasis [21,22]. In addition, circulating EMPs derived from activated endothelial cells did not contain nuclear components and they have also been shown to have pro-angiogenic and cardio-protective properties [23,24]. In opposite, apoptotic EMPs may originate from damaged endothelial cells that concentrate immune mediators, generating powerful signaling by the simultaneous receptor interaction, and they are discussed a marker of endothelial cell injury and vascular aging [25,26]. However, the potential relevance of different phenotypes of circulating EMP among MetS patients is still not understood.

The Aim of the Study

To investigate the pattern of circulating EMPs in MetS patients with CHF.

METHODS

The study retrospectively evolved 101 patients with MetS (54 subjects with CHF and 47 patients without CHF) without documented coronary artery stenosis >50% at least of one artery and 35 healthy volunteers who were examined between February 2013 and November 2013. The study was approved by the Local Ethics Committee of State Medical University, Zaporozhye, Ukraine. The study was performed in conformity with the Declaration of Helsinki. All the patients have given their informed written consent for participation in the study. MetS was diagnosed based on the National Cholesterol Education Program Adult Treatment Panel III criteria [27]. Patients were enrolled in the MetS cohort when at least three of the following components were defined: Waist circumference ≥90 cm or ≥80 cm in men and women, respectively; high-density lipoprotein cholesterol (HDL-C) <1.03 mmol/L or <1.3 mmol/L in men and women, respectively; triglycerides (TG) ≥1.7 mmol/L; BP ≥130/85 mmHg or current exposure of antihypertensive drugs; fasting plasma glucose ≥5.6 mmol/L. Subjects with defined T2DM or treatment with oral antidiabetic agents or insulin were not enrolled in the study. Current smoking was defined as consumption of one cigarette daily for 3 months. Anthropometric measurements were made using standard procedures.

Methods for Visualization of Coronary Arteries

Contrast-enhanced multispiral computed tomography angiography has been performed for all the patients with dysmetabolic disorder prior to their inclusion in the study on optima CT660 scanner (GE Healthcare, USA) using non-ionic contrast Omnipaque (Amersham Health, Ireland) [28]. Subjects with atherosclerotic lesions >50% of the diameter at least of one coronary artery were excluded for further enrollment in the study.

Transthoracic Echocardiography

Transthoracic echocardiography was performed according to a conventional procedure on ultrasound scanner ACUSON (SIEMENS, Germany) in B-mode and tissue Doppler imaging with phased probe of 2.5-5 MHz. Left ventricular (LV) end-diastolic and end-systolic volumes, LV ejection fraction (LVEF) were measured by modified Simpson's method [29].

Calculation of Glomerular Filtration Rate (GFR)

GFR was calculated with CKD-EPI formula [30].

Measurement of Circulating Biomarkers

To determine circulating biomarkers, blood samples were collected at baseline in the morning (at 7-8 a.m.) into cooled silicone test tubes wherein 2 mL of 5% Trilon B solution were added. Then they were centrifuged upon permanent cooling at 6,000 rpm for 3 min. Plasma was collected and refrigerated immediately to be stored at a temperature −70°C. Serum N-terminal brain natriuretic peptide (NT-proBNP), adiponectin, receptor activator of NF-κB ligand (RANKL), and osteoprotegerin (OPG) were measured by high-sensitive enzyme-linked immunosorbent assays using commercial kits (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany) according to the manufacturers' recommendations. The inter-assay coefficients of variation were as follows: NT-proBNP: 4.5%, adiponectin: 5%, RANKL: 7.0%; OPG: 8.2%.

High-sensitive C-reactive protein (hs-CRP) was measured by commercially available standard kit (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The intra-assay and inter-assay coefficients of variation were <5%.

Fasting insulin level was measured by a double-antibody sandwich immunoassay (Elecsys 1010 analyzer, F. Hoffmann-La Roche Diagnostics, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation were <5%. The lower detection limit of insulin level was 1.39 pmol/L.

IR was assessed by the homeostasis model assessment for IR (HOMA-IR) [31] using the following formula:

HOMA-IR (mmol/L × μU/mL) = fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5

IR was defined when estimated HOMA-IR value was over 2.77 mmol/L × μU/mL as it was defined previously [32,33].

Concentrations of total cholesterol and HDL-C were measured by fermentation method. Concentration of cholesterol of low-

density lipoproteins (LDL-C) was calculated according to the Friedewald formula (1972) [34].

Assay of Circulating EMPs

Circulating EMPs were isolated from 5 ml of venous citrated blood drawn from the fistula-free arm. To prevent contamination of samples platelet-free plasma (PFP) was separated from whole blood and then was centrifugated at 20,500 × rpm for 30 min. EMP pellets were washed with DMEM (supplemented with 10 μg/mL polymyxin B, 100 UI of streptomycin, and 100 U/ml penicillin) and centrifuged again (20,500 rpm for 30 min). The obtained supernatant was extracted, and EMP pellets were re-suspended into the remaining 200 μL of supernatant. PFP, EMPs, and the supernatant were diluted 5-, 10-, and 5-fold in phosphate-buffered saline, respectively.

Endothelial-derived apoptotic and activated microparticles were phenotyped by flow cytometry by phycoerythrin-conjugated monoclonal antibody against CD31 (platelet endothelial cell adhesion molecule-1), CD144 (vascular endothelial cadherin), CD62E (E-selectin), and annexin V (BD Biosciences, USA) followed by incubation with fluorescein isothiocyanate-conjugated annexin V (BD Biosciences, USA) per high-definition fluorescence activated cell sorter (HD-FACS) methodology independently after supernatant diluted without freeze [35]. The samples were incubated in the dark for 15 min at room temperature according to the manufacturer's instructions. For each sample, 500 thousand events have been analyzed. EMPs gate was defined by size, using 0.5 and 1.0 μm 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 [36].

Statistical Analysis

Statistical analysis of the results obtained was performed in SPSS system for Windows, Version 22 (SPSS Inc., Chicago, IL, USA). The data were presented as mean (M) and standard deviation or 95% confidence interval; as well as median (Me) and 25%-75% interquartile range. To compare the main parameters of patient cohorts, two-tailed Student's *t*-test or Shapiro–Wilk U-test were used. To compare categorical variables between groups, Chi-square test and Fisher F exact test were used. Predictors of EMPs elevation in patients were examined in simple and multiple linear regression analysis. C-statistics, integrated discrimination indices (IDI) and net reclassification improvement (NRI) were utilized for prediction performance analyses. A two-tailed $P < 0.05$ was considered as significant.

RESULTS

general characteristic of patients participating in the study was reported in Table 1. As expected, there was a significant difference between healthy volunteers and entire cohort of enrolled patients in based model (body mass index [BMI]), waist circumference, cardiovascular risk factors (hypertension, dyslipidemia, adherence to smoking), CHF class, BP levels, heart rate, LVEF, HOMA-IR, lipid abnormalities, and Framingham risk score. Therefore, CD31+/annexin V+ EMPs were significantly elevated in patient cohort, while CD62E+ EMPs and CD62E+ to CD31+/annexin V+ ratio were elevated in healthy persons when compared with MetS patients ($P < 0.001$).

MetS patients without CHF have demonstrated lower incidence of dyslipidemia, lower concentrations of LDL-C, hs-CRP, serum RANKL, OPG, NT-proBNP, and CD31+/annexin V+ EMPs when compared with MetS subjects with CHF. Therefore, higher LVEF, TG, HDL-C, and HOMA-IR were found in MetS patients without CHF in comparison to MetS patients with CHF. Interestingly, similarities of circulating levels of EMPs different origin were determined in both cohorts apart from CD31+/annexin V+ EMPs. Therefore, CD62E+ to CD31+/annexin V+ ratio was found to be higher in the MetS patients without CHF compared with MetS patients with CHF.

The simple linear regression analysis shown being correlation between activated endothelial cell-derived EMP to apoptotic-derived EMP ratio, cardiovascular risk factors, hemodynamic performances, and other biomarker. We found that CD62E+ to CD31+/annexin V+ ratio were directly related with NT-proBNP ($r = -0.512$, $P = 0.001$), BMI ($r = 0.46$, $P = 0.001$), OPG ($r = -0.412$, $P = 0.001$), hs-CRP ($r = -0.445$, $P = 0.001$), HOMA-IR ($r = 0.414$, $P = 0.001$), eGFR ($r = 0.312$, $P = 0.001$), TG ($r = 0.304$, $P = 0.001$), dyslipidemia ($r = -0.248$, $P = 0.001$), creatinine ($r = 0.242$, $P = 0.001$), gender ($r = 0.228$, $P < 0.001$ for male), age ($r = -0.225$, $P = 0.001$), and smoking ($r = -0.212$, $P = 0.001$). No significant association CD62E+ to CD31+/annexin V+ ratio with fasting plasma glucose, HbA1c, means of systolic and diastolic BP, waist circumference was found. We did not find possible age- and gender-related correlation between metabolic status and the presence of EMPs.

Using multiple linear regression analyses, independent impact of BMI ($r = 0.424$, $P = 0.003$), NT-proBNP ($r = -0.423$, $P = 0.001$), OPG ($r = -0.419$, $P = 0.001$), and hs-CRP ($r = -0.384$, $P = 0.001$) on decreased CD62E+ to CD31+/annexin V+ ratio was found.

Using C-statistics for Models with BMI and circulating biomarkers (hs-CRP, OPG and NT-proBNP) as Continuous Variables we found that adding of combination of inflammatory biomarkers (hs-CRP, OPG and NT-proBNP) to the BMI improved the relative IDI by 11.4% for decreased CD62E+ to CD31+/annexin V+ ratio [Table 2].

When we used other models constructed on entering variables categorical NRI appears to be improved up to 5% for decreased CD62E+ to CD31+/annexin V+ ratio (available for three biomarkers as continuous variables) [Table 3]. Three biomarkers (NT-proBNP, hs-CRP, OPG) improve significantly predictive model based on BMI for decreased CD62E+ to CD31+/annexin V+ ratio. In patient study population for category-free NRI, 5% of events ($P = 0.001$), and 16% of non-events ($P = 0.001$) were correctly reclassified by the addition of circulating biomarkers (NT-proBNP, hs-CRP, OPG) to the base

Table 1: General characteristic of patients participating in the study

Characteristics	Healthy volunteers (n=35)	Entire cohort of enrolled patients (n=101)	MetS patients without CHF (n=47)	MetS patients with CHF (n=54)
Age, years	46.12±4.22	48.34±7.80	48.30±3.94	48.42±6.10
males, *n* (%)	23 (65.7)	64 (63.3)	30 (63.8)	34 (63.0)
BMI, kg/m^2	21.5 (25-75% IQR=16.1-23.5)	28.4 (25-75% IQR=16.5-32.4)*	28.2 (25-75% IQR=16.7-31.0)	28.5 (25-75% IQR=16.8−32.1)
Waist circumference, sm	78 (25-75% IQR=63-89)	93 (25-75% IQR=76-103)*	92 (25-75% IQR=77-105)	95 (25-75% IQR=90−104)
Hypertension, *n* (%)	-	68 (67.3)*	32 (68.0)	36 (66.7)
I NYHA class CHF (%)	-	17 (16.8)*	-	17 (31.5)#
II NYHA class CHF (%)	-	22 (21.9)*	-	22 (40.7)#
III NYHA class CHF (%)	-	15 (14.9)*	-	15 (27.8)#
Dyslipidemia, *n* (%)	-	59 (58.4)*	26 (55.3)	33 (61.1)#
Adherence to smoking, n (%)	6 (17.1)	31 (30.7)*	16 (34.0)	15 (27.7)
Framingham risk score	2.55±1.05	8.12±2.88*	8.09±2.12	9.28±2.32
Systolic BP, mmHg	122±5	138±6*	137±4	139±5
Diastolic BP, mmHg	72±4	87±6*	87±5	88±4
Heart rate, beats per 1 min.	66±6	75±7*	71±6	78±5
LVEF, %	66.8 (95% CI=61.2-73.5)	50.6 (95% CI=42.5-55.3)*	52.4 (95% CI=48.3-57.5)	44.2 (95% CI=40.3-48.1)#
GFR, mL/min/1.73 m^2	102.1 (95% CI=91.4-113.2)	93.1 (95% CI=79.5-109.7)	92.5 (95% CI=83.1-107.4)	93.8 (95% CI=80.4-106.8)
HbA1c, %	4.75 (25-75% IQR=4.36-5.12)	6.7 (25-75% IQR=5.3-8.2)*	6.82 (25-75% IQR=5.61-8.37)	6.64 (25-75% IQR=5.53-8.31)
fasting blood glucose, mmol/L	4.52 (25-75% IQR=4.43-4.76)	6.50 (25-75% IQR=5.8-7.0)*	6.46 (25-75% IQR=5.73-6.86)	6.54 (25-75% IQR=5.69-6.98)
Insulin, μU/mL	4.98 (25-75% IQR=1.5-14.1)	15.45 (25-75% IQR=13.69-16.62)*	15.2 (25-75% IQR=12.5-15.7)	15.6 (25-75% IQR=12.9-16.8)
HOMA-IR, mmol/L×μU/mL	1.01 (25-75% IQR=0.91-1.07)	4.46 (25-75% IQR=4.17-5.20)*	4.36 (25-75% IQR=4.12-5.18)	4.53 (25-75% IQR=4.11-5.12)
creatinine, μmol/L	62.1 (95% CI=55.7-82.4)	71.2 (95% CI=59.9-87.2)	70.5 (95% CI=59.6-88.3)	72.3 (95% CI=56.1-86.9)
Total cholesterol, mmol/L	4.76 (95% CI=4.21-5.05)	5.3 (95% CI=4.6-6.0)*	5.3 (95% CI=4.5-5.9)	5.4 (95% CI=4.8-5.8)
LDL-C, mmol/L	3.10 (95% CI=2.78-3.21)	3.60 (95% CI=3.20-4.18)*	3.48 (95% CI=3.30-4.07)	3.80 (95% CI=3.20-4.20)#
HDL-C, mmol/L	1.13 (95% CI=1.05-1.17)	0.94 (95% CI=0.92-1.06)*	1.01 (95% CI=0.90-1.13)	0.94 (95% CI=0.88-1.04)
TG, mmol/L	1.18 (95% CI=1.07-1.30)	1.68 (95% CI=1.44-1.98)*	1.77 (95% CI=1.62-1.95)	1.45 (95% CI=1.42-1.51)#
hs-CRP, mg/L	4.11 (25-75% IQR=0.97-5.03)	7.96 (25-75% IQR=4.72-9.34)*	7.80 (25-75%, IQR=4.92-9.43)	8.13 (25-75% IQR=5.90-10.85)#
sRANKL, pg/mL	16.10 (25-75% IQR=2.1-30.1)	29.10 (25-75% IQR=15.2-56.7)*	24.10 (25-75% IQR=14.7-36.9)	34.20 (25-75% IQR=20.1-55.2)#
OPG, pg/mL	88.3 (25-75% IQR=37.5-136.6)	804.5 (25-75% IQR=579.9-1055.3)*	718.5 (25-75% IQR=572.1-846.2)	882.5 (25-75% IQR=697.1-1046.2)#
Adiponectin, mg/L	6.17 (25-75% IQR=3.44-10.15)	13.65 (25-75% IQR=10.12-24.93)*	13.61, (25-75% IQR=9.74-22.35)	14.12 (25-75% IQR=10.12-23.10)
NT-proBNP, pg/mL	96.1 (95% CI=64.5-125.8)	687.5 (95% CI=84.7-1244.5)*	92.2 (95% CI=55.8-133.2)	1475.3 (95% CI=584.7-2293.5)#
CD144+/CD31+EMPs, n/mL	0.87 (25-75% IQR=0.27-1.25)	0.92 (25-75% IQR=0.36-1.32)	0.89 (25-75% IQR=0.32-1.29)	0.93 (25-75% IQR=0.41-1.33)
CD144+/annexin V+EMPs, n/mL	0.95 (25-75% IQR=0.11-1.78)	1.15 (25-75% IQR=0.13-2.41)	1.08 (25-75% IQR=0.13-2.39)	1.17 (25-75% IQR=0.15-2.55)
CD144+/CD31+/annexin V+EMPs, n/mL	0.82 (25-75% IQR=0.27-1.55)	1.01 (25-75% IQR=0.39-1.70)	0.94 (25-75% IQR=0.38-1.52)	1.12 (25-75% IQR=0.40-1.67)
CD31+/annexin V+EMPs, n/mL	0.154 (25-75% IQR=0.03-0.21)	0.316 (25-75% IQR=0.261-0.374)*	0.285 (25-75% IQR=0.253-0.318)	0.355 (25-75% IQR=0.294-0.382)#
CD62E+EMPs, n/mL	1.35 (25-75% IQR=0.95-1.68)	1.03 (25-75% IQR=0.86-1.13)*	1.05 (25-75% IQR=0.88-1.18)	0.98 (25-75% IQR=0.89-1.12)
CD62E+to CD31+/annexin V+ratio, unit	8.77 (25-75% IQR=7.95-9.18)	3.26 (25-75% IQR=3.23-3.30)*	3.68 (25-75% IQR=3.47-3.81)	2.76 (25-75% IQR=2.42-3.04)#

Data are presented as mean and ±SE or 95% CI; Me and 25-75% IQR. Categorical variables are expressed as numerous (n) and percentages (%). *P* value is a comparison of mean or median variables between both cohorts (ANOVA test). *Significant difference between healthy subjects and the entire cohort of enrolled patients; #Significant difference between MetS subjects with and without CHF. CI: Confidence interval, IQR: Inter quartile range, BMI: Body mass index, TG: Triglycerides, BP: Blood pressure, CHF: Chronic heart failure, LVEF: Left ventricular ejection fraction, GFR: Glomerular filtration rate, EMPs: Endothelial-derived microparticles, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, hs-CRP: High sensitive C reactive protein, sRANKL: Serum receptor activator of NF-κB ligand, SE: Standard error, OPG: Osteoprotegerin, HOMA-IR: Homeostasis model assessment for insulin resistance, NT-proBNP: N-terminal brain natriuretic peptide, MetS: Metabolic syndrome

Table 2: C-statistics for models with BMI, NT-proBNP, hs-CRP, and OPG as continuous variables

Models	Dependent variable: CD62E+to CD31+/annexin V+ratio			
	AUC (95% CI)	ΔAUC	IDI (±SE)	Relative IDI (%)
Model 1 (based model: BMI)	0.654	-	-	-
BMI+NT-proBNP versus BMI	0.686	0.032; $P<0.05$	0.02±0.008	5.9
BMI+OPG versus BMI	0.690	0.036; $P<0.05$	0.04±0.010	5.7
BMI+NT-proBNP+OPG versus BMI	0.692	0.038; $P<0.05$	0.03±0.008	6.5
BMI+hs-CRP versus BMI	0.680	0.026; $P<0.05$	0.03±0.007	4.3
BMI+OPG+hs-CRP versus BMI	0.706	0.052; $P<0.05$	0.04±0.009	6.1
BMI+NT-proBNP+hs-CRP+OPG+versus BMI	0.710	0.056; $P<0.001$	0.03±0.009	11.4

Relative IDI – calculated as the ratio of IDI over the discrimination slope of the model 1 (BMI). AUC: Area under curve, SE: Standard error, hs-CRP: High sensitive C-reactive protein, IDI: Integrated discrimination indices, BMI: Body mass index, OPG: Osteoprotegerin, NT-proBNP: N-terminal brain natriuretic peptide

Table 3: Prediction performance analyses for models with BMI and circulating inflammatory biomarkers (NT-proBNP, hs-CRP, OPG) as continuous variables for decreased CD62E+ to CD31+/annexin V+ratio

Characteristics	Model 2 versus Model 1
Categorical NRI	0.19 (95% CI=0.15-0.22)
Percentage of events correctly reclassified	3 (P=0.12)
Percentage of non-events correctly reclassified	5 (P=0.001)
Categorical free NRI	0.34 (95% CI=0.30-0.39)
Percentage of events correctly reclassified	5 (P=0.001)
Percentage of non-events correctly reclassified	16 (P=0.001)

Model 1 - BMI; Model 2 - BMI+NT-proBNP+hs-CRP+OPG, NRI: Net reclassification improvement, hs-CRP: High sensitive C-reactive protein, BMI: Body mass index, OPG: Osteoprotegerin NT-proBNP: N-terminal brain natriuretic peptide, CI: Confidence interval

model (BMI) for decreased CD62E+ to CD31+/annexin V+ ratio.

DISCUSSION

The results of the study have clarified that patients with MetS may have different predominantly appeared phenotypes of circulating EMPs distinguished healthy volunteers. Being of impaired phenotype of circulating EMPs in study patient population is determined as imbalance between the number of EMPs with pro-angiogenic capacities (CD62E+) and EMPs delivered in resulting of apoptosis (CD31+/annexin V+). A significant decreased CD62E+ to CD31+/annexin V+ ratio was found in MetS patients in comparison with healthy volunteers. Moreover, MetS subjects with CHF demonstrated lower level of CD62E+ to CD31+/annexin V+ ratio predominantly associated with decreased CD31+/annexin V+ EMPs. Thus, a significant difference between healthy subjects and MetS patients enrolled in the study regarding CD62E+ to CD31+/annexin V+ ratio may probably reflect impaired phenotype of EMPs with surpassed apoptotic-labeled microparticles. CHF in MetS patient population did not significantly effect on processes of endothelium activation, although apoptosis-related forming of EMPs is exaggeratedly increased.

We suggest that the ability of the endothelium to preserve a secretion of pro-angiogenic EMPs among MetS patients may have a causality role in improving clinical outcomes in MetS subjects with CHF in comparison to none-MetS subjects with cardiac failure. Leroyer *et al*. [37] believe apoptotic EMPs produced due to inversion of the lipid membrane during apoptosis are secondary messengers contributed to vascular injury and tissue damage in diabetes. The interrelationship between apoptotic EMPs and vascular complications of T2DM is closely expected, despite microvesicles that are phenotypically nearly identical to CD31+/annexin V+ EMPs were not elevated in dysmetabolic disorders without existing atherosclerosis and cardiovascular complications [22,25,38]. In the study, we found that CD62E+ to CD31+/annexin V+ ratio might be referred as object characterized predominantly immune phenotype of circulating EMPs. According the opinion of many investigators, elevated apoptotic EMPs levels reflect cellular injury and appear to be a surrogate marker of vascular dysfunction [24,25,38,39]. Moreover, apoptotic-derived EMPs play a pivotal role in the development of vascular complications in dysmetabolic diseases for they stimulate pro-inflammatory responses in target cells and promote various processes, such as coagulation, thrombosis, angiogenesis, and neovascularization [40,41]. These findings support our hypothesis that elevated EMPs are associated with several cardiovascular risk factors and MetS. Probably there is a more significant independent risk factor than length of impaired glucose level, diabetic disease, or the presence of hypertension [42-44]. In contrast, activated endothelial cell-derived microparticles may avoid inducing tissue injury and worsening vasomotion via genome involved mechanisms, and they are thereby able to protect the endothelium from damage [45]. Although it has been continued to emphasize that apoptotic subpopulation of EMPs are elevated in metabolic disorders, we did not found significant differences in circulating EMPs labeled as CD144+/annexin V+, CD144+/CD31+/annexin V+, and CD144+/CD31+, except CD31+/annexin V+, and CD62E+ between healthy volunteers and patients with metabolic disorders without existing atherosclerosis. Recent clinical studies shown increased CD31+/annexin V+ EMPs to CD62E+ EMPs ratio in dysmetabolic persons, i.e. T2DM [46]. Therefore, there was a significant association between CD31+/annexin V+ EMPs to CD62E+ EMPs ratio and circulating level of pro-inflammatory cytokines (hs-CRP, OPG, and adiponectin) that are suitable for both T2DM and MetS. Surprisingly, in our study, independent associations of CD62E+ to CD31+/annexin V+ ratio with cardiovascular risk factors were not found, while association TG and lipid abnormality with CD62E+

to CD31+/annexin V+ ratio was shown. Recent study have been shown that dyslipidemia and especially increased TG level in MetS patient populations may negatively effect on the ability of the endothelium to produce activated microvesicles with angiogenic capacities and secreted apoptotic-derived microparticles [47,48]. Therefore, it is discussed the question regarding dyslipidemia-induced apoptotic-related EMPs production [48]. In fact, infiltration of subintima by LDL may induce the production of free radicals that is crucial for oxidation of cytoskeleton and membrane proteins of endothelial cells. Membrane vesiculation is considered a mechanism of elimination of oxidative stress products including carbonylated proteins, oxidized lipoproteins, intermediates, etc. [49]. Therefore, this oxidative-driven vesiculation of endothelial cells may relate to low intensity inflammation in vasculature, which associate with over production of cytokines i.e. hs-CRP, adiponectin, and OPG [50,51]. Moreover, membrane vesiculation may enhance inflammatory cytokines in convey of biomechanical stress [51]. As well-known hs-CRP and OPG appear to be sufficiently increased in MetS and they may be compensatory up-regulated in the atherosclerosis and, however, in vascular inflammation [52]. Less known about innate mechanism regarding NT-proBNP-dependent regulation of microvesiculation in endocardial endothelium. The clinical significance of this phenomenon is still not clear and planned/ongoing clinical studies with large sample population are absent. Whether NT-proBNP is caused to imbalance between EMP subsets is not understood. Here, we report that MetS patients with CHF, who have not angiographic evidence of clinically significant atherosclerosis, may distinguish in inflammatory pattern of circulating EMPs and that these differences are more much sufficient than adipocytokine profile and glucose impairment. Although initially there is skepticism regarding origin of imbalance of activated and apoptotic EMP numerous in patients with impaired glucose metabolism and dyslipidemia, we suppose that inflammatory cytokine overproduction and probably lipid abnormalities may consider a possible cause of predominantly immune phenotype of EMPs not directly related with glucose impairment. Indeed, there are evidences regarding being of paracrine and endocrine regulation of lipid storage and cell size of white adipocytes by specific micro-RNAs derived by EMPs in MetS [53]. Obviously patients with different types of dysmetabolic disorders might have different inflammatory pattern of EMPs, which contribute to the development of cardiovascular complications, such as CHF. Collectively, there are raised reports regarding that the presence and number of single EMP population is not obligatory object reflected cardiovascular risk, while predominant immune phenotype is probably. Overall, determination of predominantly immune phenotype of EMPs appears to be attractive for risk classification and probably creating individualized prediction score in MetS patients, because of circulating level of pro-inflammatory cytokines demonstrates a high biological variability [54]. On the other hand, EMP determination is not easy for use and pre-analytical and analytical errors are frequently appeared [55]. Finally, determination of inflammatory phenotype of EMPs encompasses responses of CVD risk factors and documented CVD appear to be attractive and it is required more efforts to understand whether the novel biological marker is useful or not.

Study Limitations

This study has some limitations. All enrolled subjects were comparable to age and sex because of the design of the study was retrospective. This is a limitation appears to be serious for determination of possible age- and sex-related association regarding numerous of circulating microparticles. The results of our study do not allow producing any issues regarding this. Measuring of LDL-C level by Friedewald formula is limitation too. 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. We used PFP to prevent of contamination of samples with microparticles originated from platelets. Therefore, preparation of microparticle isolates from samples is the most sophisticated step for further examination. There were several technical-related difficulties in the measurement of EMPs. In fact, lack of the standard protocol for isolating and detecting circulating EMPs obtained from the plasma. According to the opinion of the majority experts, centrifugation become the main factor-mediated reliability of the EMP determination in samples and contributed to biological variability of EMP count. The removal of all cells from blood may be pre-analytical challenging where small apoptotic bodies overlap in size with other types of EMPs. Although HD-FACS methodology is widely used, theoretically overlap between two or more fluorochromes might reflect some obstacles for further interpretation of obtained results. Therefore, the analytical level for determination EMP size (taken into consideration the equipment and methodology) was defined as 300 nm, despite low size microparticles may be detected with other methods, such as nanoparticle tracking. Probably, another result regarding numerous of circulating apoptotic-derived EMPs could be obtained when other equipment used. Currently, nanoparticle tracking analysis, flow cytometry, and other methods of identification of EMPs are not affordable in routine clinical practice. In future, the balance and the intensity of endothelial damage, integrity and tissue repair reflected by changes in circulating EMPs and other types of pro-angiogenic progenitor cells might be open the novel approaches to assay the risk of CVD and identify vulnerable population before therapeutic care.

Another limitation of the present study is that a specific role of EMPs is also possible and has not been characterized in depth in T2DM patients. However, the authors suppose that these restrictions might have no significant impact on the study data interpretation. In addition, retrospective, relative small sample size may limit the significance of the present study. However, taken together these data are very promising, and they are required new investigations with higher statistical power and increased sample size to be overcome the internal limitations of the study.

CONCLUSION

We found that biomarkers of biomechanical stress (NT-proBNP) and inflammation (hs-CRP, OPG) remain statistically significant predictors for decreased CD62E+ to CD31+/annexin V+ ratio in MetS patients with CHF.

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AUTHORS' CONTRIBUTIONS

Alexander E. Berezin initiated the hypothesis and designed the study protocol, contributed to collect, analyze, and interpret the data, performed statistical analysis, wrote the manuscript. Alexander A. Kremzer contributed to enroll the patients; collected and analyzed the data, reviewed the source documents, drafted a paper. Tatyana A. Samura contributed to the study protocol design, performed visualization procedures, analyzed the results of examinations, and drafted a paper. Tatyana A. Berezina contributed to the study protocol design, enrolled the patients in the study, collected the data, analyzed, and interpreted the data obtained and drafted a paper. All authors revised the manuscript critically, had consolidated agreement to be accountable for all aspects of the work, and final approved of the version to be published.

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