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Pattern of circulating microparticles in chronic heart failure patients with metabolic syndrome: Relevance to neurohumoral and inflammatory activation

Alexander E. Berezin [a,*], Alexander A. Kremzer [b], Tatyana A. Berezina [c], Yulia V. Martovitskaya [d]

[a] Internal Medicine Department, State Medical University, 26, Mayakovsky av., Zaporozhye Postcode 69035, Ukraine
[b] Clinical Pharmacology Department, State Medical University, Zaporozhye, Ukraine
[c] Private Center "Vita-Center", Zaporozhye, Ukraine
[d] Pathology Department, State Medical University, Zaporozhye, Ukraine

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ABSTRACT

Background: The role of pattern of circulating endothelial cell-, platelet-, and monocyte-derived microparticles in metabolic syndrome (MetS) patients with chronic heart failure (CHF) is not still understood.
The aim of the study was to investigate a pattern of circulating MPs in MetS patients with CHF in relation to neurohumoral and inflammatory activation.
Methods: The study retrospectively involved 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 MPs were phenotyped by flow cytometry technique.
Results: The results of the study have shown that numerous of the circulating platelet-derived and monocyte-derived MPs in subjects with MetS (with or without CHF) were insufficiently distinguished from the level obtained in healthy volunteers. We found an elevated level of CD31 +/annexin V+ MPs in association with a lower level of CD62E+ MPs. All these led to decreased CD62E+ to CD31 +/annexin V+ ratio among patients with MetS in comparison with healthy volunteers, as well as in MetS patients with CHF compared with those who did not demonstrated CHF. Therefore, we found that biomarkers of biomechanical stress (NT-proBNP) and inflammation (hs-CRP, osteoprotegerin) remain statistically significant predictors for decreased CD62E+ to CD31 +/annexin V+ ratio in MetS patients with CHF.
In conclusion, decreased CD62E+ to CD31 +/annexin V+ ratio reflected impaired immune phenotype of MPs may be discuss surrogate marker of CHF development in MetS population.

1. Introduction

The traditionally recognized metabolic syndrome (MetS) is defined as risk factor clustering related to the development of type 2 diabetes mellitus (T2DM) and cardiovascular disease [1]. MetS includes abdominal obesity, insulin resistance, dyslipidemia, and elevated blood pressure and associates with other co-morbidities including the prothrombotic and proinflammatory states [2]. Accumulating evidences have been shown that MetS is a powerful risk factor for cardiovascular disease (CVD) event, as well as all-cause and CVD mortality in total population [3–5]. The underlying pathophysiological mechanisms resulting in the MetS, i.e. insulin resistance (IR), associate with activation of neurohumoral mechanisms, immunity, cytokine production, systemic pro-inflammatory response, and oxidative stress [6–8]. All these factors may affect the development of CVD through inducing endothelial dysfunction [9–10] and microvascular inflammation [11].

Recent studies have shown a controversial role of MetS in patients at high risk of chronic heart failure (CHF) and in subjects with documented CHF. Although MetS associates with cardiovascular risk factors and CVD outcomes [12–15], prognostic impact of MetS on CHF progression is not fully confirmed and widely discussed [16,17]. Therefore, it is still unclear whether MetS may induce development and progression of cardiac failure through imbalance between endothelial injuries and repair [18,19]. Probably microparticles (MPs) corresponding to cell-to-cell cooperation, immunity, tissue reparation, and vascular function, are key factors that coordinate microvascular integrity and function [20].

Extracellular microparticles are microvesicles with sizes ranging between 50 and 1000 nm released from the plasma membrane of a wide variety of cells, including endothelial cells, mononuclear cells, and platelets, by specific (cytokine stimulation, apoptotic agents, mononuclear

* Corresponding author.
E-mail address: dr_berezin@mail.ru (A.E. Berezin).

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cooperation, coagulation, etc.) and non-specific (shear stress) stimuli [21]. Circulating endothelial-derived microparticles (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, as well as proteins and lipid components, from one cell to another without direct cell-to-cell contact to maintain cell homeostasis [22,23]. EMPs derived from activated endothelial cells may have pro-angiogenic and cardio-protective properties [24]. In opposite, apoptotic EMPs that originated from damaged endothelial cells are discussed as marker of endothelial cell injury and vascular aging [25].

Platelet-derived microparticles (PMPs) are a heterogeneous population of microvesicles that are secreted from chemokine and cytokine activated platelets. PMPs that mediate multiple cellular responses predominantly affected protein and lipid metabolism, coagulation and inflammation [26]. Elevated PMPs show a relation to clinical outcomes and mortality in several patient populations [27].

Numerous studies have shown that monocyte-derived microparticles (MMPs) are realized from activated and/or apoptotic monocytes in response to various stimuli, i.e. antigen stimulation, growth factors, inflammatory interleukins, and chemokines and cytokines [28–30]. Elevated levels of circulating MMPs are documented in almost all thrombotic diseases, infective, rheumatic and autoimmune diseases, stroke, myocardial infarction, atrial fibrillation, as well as in metabolic, ischemic/hypoxic states, and critical conditions [31–33]. However, the significance of MPs in MetS patients as an inductor of development and progression of CHF remains controversial. An example of this controversy is that it is still unknown if circulating MPs found in peripheral blood cause injury of the endothelium and worsening of CHF whether they are the result of disease progression in response to endothelial dysfunction and vascular dysintegrity [34,35]. The aim of the study was to investigate the pattern of circulating endothelial cell-, platelet-, and monocyte-derived MPs in MetS patients with CHF in relation to neurohumoral and inflammatory activation.

2. 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 the 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 [36]. 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 (HDL) cholesterol <1.03 mmol/L or <1.3 mmol/L in men and women respectively; triglycerides ≥1.7 mmol/L; blood pressure ≥130/85 mm Hg or current exposure of antihypertensive drugs; and 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 three months. Anthropometric measurements were made using standard procedures.

2.1. 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) [37]. Subjects with atherosclerotic lesions >50% of diameter at least of one coronary artery were excluded for further enrollment in the study.

2.2. 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 [38].

2.3. Calculation of glomerular filtration rate

Glomerular filtration rate (GFR) was calculated with the CKD-EPI formula [39].

2.4. 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 was added. Then they were centrifuged upon permanent cooling at 6000 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, 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.

Insulin resistance was assessed by the homeostasis model assessment for insulin resistance (HOMA-IR) [40] using the following formula:

$$\text{HOMA-IR (mmol/L} \times \mu\text{U/mL)} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin } (\mu\text{U/mL})/22.5.$$

Concentrations of total cholesterol (TC), cholesterol of low-density lipoproteins (LDL-C) and cholesterol of high-density lipoproteins (HDL-C) were measured by enzymatic method.

2.5. Assay of circulating endothelial-derived microparticles

Circulating MPs 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. PFP was centrifugated at 20,500 × rpm for 30 min. MP pellets were washed with DMEM (supplemented with 10 μg/mL of polymyxin B, 100 UI of streptomycin, and 100 U/ml of penicillin) and centrifuged again (20,500 rpm for 30 min). The obtained supernatant was extracted, and MP pellets were re-suspended into the remaining 200 μL of supernatant. PFP, MPs, and supernatant were diluted five-, 10-, and five-fold in PBS, respectively.

MPs were labeled and characterized by flow cytometry by phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (platelet endothelial cell adhesion molecule [PECAM]-1), CD41a, CD64, CD105, CD144 (vascular endothelial [VE]-cadherin), CD62E (E-selectin), and Annexin V (BD Biosciences, USA) followed by incubation with fluorescein isothiocyanate (FITC)-conjugated Annexin V (BD Biosciences, USA) per HD-FACS (High-Definition Fluorescence Activated Cell Sorter) methodology independently after supernatant diluted without freeze [41]. The samples were incubated in the dark for 15 min at room temperature according to the manufacturer's instructions. The analysis of area, height, and width forward scatter (FSC) and side scatter (SSC) parameters as well as side scatter width (SSC-W) was performed. The gate for MPs was defined by size, using 0.5 and 1.0 μm beads (Sigma, St. Louis, MO, USA). For each sample, 500 thousand events have been analyzed. Compensation tubes were used with similar reagents as were used in the sample tubes. Data were constructed as numerous MPs depend on marker presentation (positive or negative) and determination of MP populations.

2.6. Determination of MP populations

CD41a+ was used as a more specific marker of platelets, and CD64+ was considered a more specific marker of monocytes. CD31 antigen was determined as essential marker for endothelial cells, platelets, and leukocytes. CD144+ was used to identify a pure population of endothelial cells. CD31+/annexin V+ and CD144+/CD31+/annexin V+ microparticles were defined as apoptotic endothelial cell-derived MPs, MPs labeled for CD105+ or CD62E+ were determined as MPs produced due to activation of endothelial cells [42].

2.7. Statistical analysis

Statistical analysis of the results obtained was performed in the SPSS system for Windows, Version 22 (SPSS Inc., Chicago, IL, USA). The data were presented as mean (M) and standard deviation (±SD); as well as median (Me) and 25%–75% interquartile range (IQR). To compare the main parameters of patient cohorts, two-tailed Student t-test or Mann–Whitney U-test was used. To compare categorical variables between groups, Chi^2 test (χ^2) and Fisher exact test were used. Univariable and multivariable regression analysis was used for determination of predictors of decreased CD62E+ to CD31+/annexin V+ ratio. All sufficient predictors with P value <0.2 obtained by univariable regression analysis were included in the multivariate regression model. A two-tailed probability value of <0.05 was considered as significant.

3. Results

General characteristic of patients participating in the study was reported in Table 1. There was a significant difference between healthy volunteers and entire patient cohort in BMI, waist circumference, cardiovascular risk factors (hypertension, dyslipidemia, adherence to smoking), CHF class, blood pressure levels, heart rate, LVEF, HOMA-IR, lipid abnormalities, and Framingham risk score. However, MetS patients without CHF have demonstrated lower incidence of dyslipidemia, lower concentrations of LDL-C, hs-CRP, sRANKL, osteoprotegerin, NT-proBNP 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.

Table 2 reports numbers of circulating MPs in patients participating in the study. Numerous of platelet-derived and monocyte-derived MPs

Table 1
General characteristic of patients participating in the study.

	Healthy volunteers (n = 35)	Entire cohort of enrolled MetS 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 (16.1–23.5)	28.4 (16.5–32.4)*	28.2 (16.7–31.0)	28.5 (16.8–32.1)
Waist circumference, sm	78 (63–89)	93 (76–103)*	92 (77–105)	95 (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, mm Hg	122 ± 5	138 ± 6*	137 ± 4	139 ± 5
Diastolic BP, mm Hg	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 (61.2–73.5)	50.6 (42.5–55.3)*	52.4 (48.3–57.5)	44.2 (40.3–48.1)#
GFR, mL/min/1.73 m^2	102.1 (91.4–113.2)	93.1 (79.5–109.7)	92.5 (83.1–107.4)	93.8 (80.4–106.8)
HbA1c, %	4.75 (4.36–5.12)	6.7 (5.3–8.2)*	6.82 (5.61–8.37)	6.64 (5.53–8.31)
Fasting blood glucose, mmol/L	4.52 (4.43–4.76)	6.50 (5.8–7.0)*	6.46 (5.73–6.86)	6.54 (5.69–6.98)
Insulin, μU/mL	4.98 (1.5–14.1)	15.45 (13.69–16.62)*	15.2 (12.5–15.7)	15.6 (12.9–16.8)
HOMA-IR, mmol/L × μU/mL	1.01 (0.91–1.07)	4.46 (4.17–5.20)*	4.36 (4.12–5.18)	4.53 (4.11–5.12)
Creatinine, μmol/L	62.1 (55.7–82.4)	71.2 (59.9–87.2)	70.5 (59.6–88.3)	72.3 (56.1–86.9)
Total cholesterol, mmol/L	4.76 (4.21–5.05)	5.3 (4.6–6.0)*	5.3 (4.5–5.9)	5.4 (4.8–5.8)
LDL-C, mmol/L	3.10 (2.78–3.21)	3.60 (3.20–4.18)*	3.48 (3.30–4.07)	3.80 (3.20–4.20)#
HDL-C, mmol/L	1.13 (1.05–1.17)	0.94 (0.92–1.06)*	1.01 (0.90–1.13)	0.94 (0.88–1.04)
TG, mmol/L	1.18 (1.07–1.30)	1.68 (1.44–1.98)*	1.77 (1.62–1.95)	1.45 (1.42–1.51)#
hs-CRP, mg/L	4.11 (0.97–5.03)	7.96 (4.72–9.34)*	7.80 (4.92–9.43)	8.13 (5.90–10.85)#
sRANKL, pg/mL	16.10 (2.1–30.1)	29.10 (15.2–56.7)*	24.10 (14.7–36.9)	34.20 (20.1–55.2) #
Osteoprotegerin, pg/mL	88.3 (37.5–136.6)	804.5 (579.9–1055.3)*	718.5 (572.1–846.2)	882.5 (697.1–1046.2) #
Adiponectin, mg/L	6.17 (3.44–10.15)	13.65 (10.12–24.93)*	13.61 (9.74–22.35)	14.12 (10.12–23.10)
NT-proBNP, pg/mL	96.1 (64.5–125.8)	687.5 (84.7–1244.5)*	92.2 (55.8–133.2)	1475.3 (584.7–2293.5)#

Note: Data are presented as mean and ±SE; median and 25–75% IQR. Categorical variables are expressed as numerous (n) and percentages (%). P-value is a comparison of mean or median variables (ANOVA test).
Abbreviations: * — significant difference between healthy subjects and entire cohort of enrolled MetS patients; # — significant difference between MetS subjects with and without CHF; SE — standard error; IQR — inter quartile range; BMI — body mass index, TG — triglycerides, BP — blood pressure, BMI — body mass index, CHF — chronic heart failure; LVEF — left ventricular ejection fraction, GFR — glomerular filtration rate, 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.

Table 2
Numbers of microparticles in participators of the study.

Immune phenotype of MPs	Healthy volunteers (n = 35)	Entire cohort of enrolled MetS patients (n = 101)	MetS patients without CHF (n = 47)	MetS patients with CHF (n = 54)
CD41a + MPs, n/μL	23 (19–28)	25 (16–33)	23 (15–31)	27 (19–36)
CD64 + MPs, n/μL	3.9 (3.5–4.6)	4.2 (3.2–5.1)	4.0 (3.4–4.8)	4.3 (3.6–5.2)
CD62E + MPs, n/μL	1.35 (0.95–1.68)	1.03 (0.86–1.13)*	1.05 (0.88–1.18)	0.98 (0.89–1.12)
CD105E + MPs, n/μL	2.32 (1.92–2.56)	2.24 (1.85–2.41)*	2.37 (1.92–2.68)	2.09 (1.58–2.50)
CD144 + MPs, n/μL	0.29 (0.22–0.36)	0.33 (0.24–0.39)	0.30 (0.22–0.37)	0.35 (0.21–0.40)
CD144 +/CD31 + MPs, n/μL	0.87 (0.27–1.25)	0.92 (0.36–1.32)	0.89 (0.32–1.29)	0.93 (0.41–1.33)
Annexin V + MPs, n/μL	4655 (3724–6237)	5495 (3988–6957)	5114 (3695–6547)	5844 (4213–7167)
CD144 +/annexin V + MPs, n/μL	0.95 (0.11–1.78)	1.15 (0.13–2.41)	1.08 (0.13–2.39)	1.17 (0.15–2.55)
CD144 +/CD31 +/annexin V + MPs, n/μL	0.82 (0.27–1.55)	1.01 (0.39–1.70)	0.94 (0.38–1.52)	1.12 (0.40–1.67)
CD31 +/annexin V + MPs, n/μL	0.154 (0.03–0.21)	0.316 (0.261–0.374)*	0.285 (0.253–0.318)	0.355 (0.294–0.382)#
CD62E + to CD31 +/annexin V + ratio, unit	8.77 (7.95–9.18)	3.26 (3.23–3.30)*	3.68 (3.47–3.81)	2.76 (2.42–3.04)#
CD105E + to CD31 +/annexin V + ratio, unit	15.1 (8.59–23.4)	7.07 (4.85–10.90)	8.31 (6.02–10.65)	5.89 (4.11–7.67)

Note: Data are presented as median and 25–75% IQR. P-value is a comparison of mean or median variables between both cohorts (ANOVA test). * — significant difference between healthy subjects and entire cohort of enrolled patients; # — significant difference between MetS subjects with and without CHF.
Abbreviations: IQR — inter quartile range; MPs — microparticles.

with immune phenotypes labeled as CD41a + and CD64 + were similar in healthy volunteers and entire patient cohort. Controversially, there is lower circulating level of activated endothelial cell-derived MPs with phenotype CD62E + and CD105E + in MetS patients compared with healthy volunteers ($P < 0.001$ for all cases). There were no significant differences between numbers of circulating MPs labeled as CD144 + and CD144 +/CD31 + originated from endothelial cells obtained from healthy volunteers and MetS patients. Although circulating levels of Annexin V +, CD144 +/annexin V +, and CD144 +/CD31 +/annexin V + MPs derived from apoptotic cells including endothelial cells were similar in both cohorts, CD31 +/annexin V + MPs were significantly elevated in MetS patient ($P < 0.001$). CD62E + to CD31 +/annexin V + ratio was significantly elevated in healthy persons when compared with MetS patients ($P < 0.001$), while CD105E + to CD31 +/annexin V + ratio was not. Interestingly, similarities of circulating levels of MPs different origin were determined in both MetS patient cohorts apart from CD31 +/annexin V + MPs. Therefore, CD62E + to CD31 +/annexin V + ratio was found to be higher in the MetS patients without CHF compared with MetS patients with CHF.

There was correlation between CD62E + to CD31 +/annexin V + ratio, cardiovascular risk factors, hemodynamic performances, and other biomarkers. We found that CD62E + to CD31 +/annexin V + ratio were directly related with NT-proBNP ($r = -0.512$, $P = 0.001$), BMI ($r = 0.46$, $P = 0.001$), osteoprotegerin ($r = -0.412$, $P = 0.001$), 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 ($r = -0.242$, $P = 0.001$), Framingham risk score ($r = -0.23$, $P = 0.001$), waist circumference ($r = 0.23$, $P < 0.001$), 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 was found. We did not find possible age- and gender-related correlation between metabolic status and the presence of EMPs.

By multivariate regression analyses, NT-proBNP (B coefficient = −0.42, P = 0.012), osteoprotegerin (B coefficient = −0.32, P = 0.026), hs-CRP (B coefficient = −0.21, P = 0.044) and BMI (B coefficient = 0.142, P = 0.036) were found as independent factors to decrease CD62E + to CD31 +/annexin V + ratio (Table 3).

4. Discussion

The results of the study have shown that numerous of the circulating platelet-derived and monocyte-derived MPs in subjects with MetS (with or without CHF) were insufficiently distinguished from level obtained in healthy volunteers. We found elevated level of apoptotic endothelial cell-derived MPs labeled CD31 +/annexin V + MPs in association with lower level of activated endothelial cell-derived MPs phenotyped as CD62E + MPs. All these led to decreased CD62E + to CD31 +/annexin V + ratio among patients with MetS in comparison with healthy volunteers, as well as in MetS patients with CHF compared with those who did not demonstrate CHF. Thus, development of CHF in MetS patients was closely related to altered balance between activated endothelial cell-derived MPs and apoptotic endothelial cell-derived MPs. This phenomenon was described as impaired phenotype of circulating MPs that might probably pre-exist CHF and appear to be before clinically significant endothelial dysfunction [20]. Whether impaired phenotype of endothelial-derived MPs is result of early stages of endothelial injury due to neurohumoral and inflammatory activation associated with dysmetabolic states or CHF development or circulating MPs are able directly induce endothelial dysintegrity is still not fully clear [35,43].

Indeed, that ability of the endothelium to release activated endothelial cell-derived MPs with pro-angiogenic capacity may have a causal role in improving clinical outcomes in CHF subjects with known MetS in comparison to none-MetS subjects [43]. Interestingly, circulating numbers of MPs that are phenotypically nearly identical to CD31 +/annexin V + MPs, were closely associated with cardiovascular risk factors, while they were not elevated in dysmetabolic disorders without known atherosclerosis or/and cardiovascular diseases [44–46]. Probably, subpopulations of MPs labeled as annexin V + are not sensitive markers of early endothelial injury and this requires performing measurements of double- and triple-labeled annexin V + MPs, such as CD31 +/annexin V + MPs. The results of the study report that

Table 3
Univariable and multivariable associations with decrease of CD62E + to CD31 +/annexin V + ratio.

	Univariable analysis		Multivariable analysis	
	B coefficient	P value	B coefficient	P value
Framingham risk score, %	−0.014	0.34	–	–
eGFR	0.012	0.22	–	–
HOMA-IR	0.018	0.26	–	–
Waist circumference	0.052	0.38	–	–
BMI	0.16	0.046	0.142	0.036
NT-proBNP	−0.46	0.001	−0.42	0.012
osteoprotegerin	−0.36	0.001	−0.32	0.026
hs-CRP	−0.28	0.001	−0.21	0.044
adiponectin	−0.015	0.22	–	–
TG	−0.032	0.42	–	–
creatinine	−0.025	0.36	–	–

Notes: The multivariate regression model included all variables with P value <0.2.
Abbreviations: BMI — body mass index; eGFR — estimated glomerular filtration rate; HOMA-IR — homeostasis model assessment for insulin resistance; NT-proBNP — N-terminal pro-brain natriuretic peptide; hs-CRP — high sensitive C-reactive protein; TG — triglycerides.

numerous of CD31 +/annexin V + MPs are not only elevated in MetS patients, but they increase sufficiently in CHF development in MetS population. Therefore, NT-proBNP, osteoprotegerin, hs-CRP and BMI independently predicted decrease of CD62E + to CD31 +/annexin V + ratio reflected impaired immune phenotype in MetS with and without CHF.

We suggested that decreased CD62E + to CD31 +/annexin V + ratio and probably elevated apoptotic endothelial cell-derived MP level may discuss surrogate markers of vascular dysfunction at early stages in MetS patients with high risk of CHF development. In fact, apoptotic endothelial cell-derived MPs play a pivotal role in the development of vascular complications in MetS and diabetes through promoting various processes, i.e. coagulation, thrombosis, angiogenesis [46,47]. 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. Therefore, platelet- and leukocyte-derived MPs have probably no sufficient effect on vascular integrity and vascular complications among MetS [48]. These findings support our hypothesis that imbalance between activated and apoptotic endothelial cell-derived MPs may have predicted value for [49,50].

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. A recent study has shown that dyslipidemia and especially increased TG level in MetS patient populations may negatively affect the ability of endothelium to produce activated microvesicles with angiogenic capacities and secreted apoptotic-derived microparticles [51,52]. Therefore, the question regarding dyslipidemia-induced apoptotic-related endothelial-derived microparticle production [53] is discussed. In fact, infiltration of the subintima by low-density lipoproteins may induce production of free radicals, oxidation of cytoskeleton and membrane vesiculation of endothelial cells [54]. The oxidative-driven vesiculation of endothelial cells may relate to low intensity inflammation in vasculature, which associates with overproduction of cytokines i.e. hs-CRP, adiponectin, and osteoprotegerin [55]. Moreover, membrane vesiculation may enhance inflammatory cytokines in conveying of biomechanical stress [56]. As well known hs-CRP and osteoprotegerin appear to be sufficiently increased in MetS and they may be compensatorily up-regulated in the atherosclerosis and in microvascular inflammation [57]. Therefore there is NT-proBNP-dependent regulation of microvesiculation in endocardial endothelium [58]. The clinical significance of this phenomenon is still not clear and planned/ongoing clinical studies with large sample population are absent [59].

Although initially there is skepticism regarding the origin of imbalance of activated and apoptotic endothelial-derived MP 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 MPs not directly related with glucose impairment. Obviously, patients with different types of dysmetabolic disorders might have different patterns of MPs [60], which contribute to the development of CHF [61,62]. Finally, determination of impaired phenotype of endothelial cell-derived MPs appears to be as novel biological marker of CHF development in MetS population.

5. Study limitations

This study has some limitations. The fist limitation is lack of standardization of MP measurements, while commercial flow cytometers exist. It is necessary to note that a large pool of MPs might be produced after blood sampling due to destruction of platelets and blood cells. In this study we used platelet free plasma to prevent contamination of samples with MPs that originated from platelets. Therefore, preparation of MP isolates from samples is the most sophisticated step for further examination. The next limitation might relate to complicated assay and suffers from resolution of MP detection technique that is worth considering. Indeed, there were several technical-related difficulties in the measurement of MPs affected centrifugation of samples, labeling of MPs, using HD-FACS methodology and final assay of results obtained. Overall the definition of a blood MP using flow cytometry is still an area of great debate. However, flow cytometry is a commonly used procedure. Although HD-FACS methodology is widely used, theoretical 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 MPs is also possible and has not been characterized in depth in MetS patients. However, the authors suppose that these optionally technical restrictions might have no significant impact on the study data interpretation. Additionally, retrospective, relative small sample size may limit the significance of the present study.

In conclusion, decreased CD62E + to CD31 +/annexin V + ratio reflected impaired immune phenotype of MPs might discuss a surrogate marker of CHF development in MetS population. Biomarkers of biomechanical stress (NT-proBNP) and inflammation (hs-CRP, osteoprotegerin) were found significant predictors for decreased CD62E + to CD31 +/annexin V + ratio in MetS patients especially with CHF.

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. Berezina contributed to the study protocol design, enrolled the patients in the study, collected the data, analyzed and interpreted of the data obtained and drafted a paper. Yulia V. Martovitskaya has performed biomarker measurements, including determination of MPs, analyzed and interpreted of the data received by flow cytometry. 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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Conflict of interests

Not declared.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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