Interrelationship between insulin resistance and impaired immune phenotype of circulating endothelial-derived microparticles in none-diabetic patients with chronic heart failure

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Abstract

Background: The causality role of different immune phenotype in IR developing among Chronic Heart Failure (CHF) subjects has not determined obviously. The aim of the study was to assess relationship between IR and immune phenotype of circulating endothelial-derived microparticles (EMPs) in patients with CHF.

Methods: The study retrospectively involved 300 CHF patients aged 48 to 62 years who were undergone multispiral computed tomography angiography or coronary angiography. All the patients have given written informed consent for participation in the study. Biomarkers were measured at baseline of the study.

Results: There were not significant differences between both cohort patients in EMPs labeled as CD144+/CD31+, CD144+/annexin V+, and CD62E+ microparticles. Higher concentrations of CD144+/CD31+/annexin V+ EMPs and CD31+/annexin V+ EMPs were found in IR subjects when compared with none IR patients. Using multivariate logistic regression analyses, we found that HOMA-IR (OR=1.14, 95% CI=1.08-1.21, P=0.001), NT-proBNP (OR=1.07, 95% CI=1.04-1.10, P<0.001), hs-CRP (OR=1.04, 95% CI=1.02-1.07, P=0.001), and NYHA class (OR=1.03, 95% CI=1.01-1.05, P<0.001) were predictors for increased CD31+/annexin V+ EMPs. Therefore, HOMA-IR (OR=1.10, 95% CI=1.05-1.17, P=0.001), NT-proBNP (OR=1.08, 95% CI=1.04-1.12, P=0.001), and NYHA class (OR=1.05, 95% CI=1.02-1.09, P=0.001) significantly predicted elevation of CD144+/CD31+/annexin V+ EMPs. Using C-statistics for Models with HOMA-IR, NYHA class, and circulating biomarkers (hs-CRP, NT-proBNP) as Continuous Variables we found that adding of combination of these biomarkers to the based model constructed with HOMA-IR did not improve the relative IDI for increased CD144+/CD31+/annexin V+ and CD31+/annexin V+ microparticles. When we used other model constructed on entering variables, IDI avoids to be improved for increased CD144+/CD31+/annexin V+ and CD31+/annexin V+ microparticles.

Conclusion: We found that IR remains statistically significant predictor for increased apoptotic-derived EMPs labelled as CD144+/CD31+/annexin V+ and CD31+/annexin V+ EMPs in none-diabetic patients with CHF patients and that these findings reflect exiting impaired phenotype of circulating EMPs in this patient population.

Abbreviations: AUC: area under curve BMI: body mass index; BMP: brain natriuretic peptide; CI – confidence interval; CHF: chronic heart failure; EMPs: endothelial-derived microparticles; IR: insulin resistance; hs-CRP: high sensitive C-reactive protein; GFR: glomerular filtration rate; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; OR: odds ratio NRI: net reclassification improvement

Introduction

Chronic Heart Failure (CHF) is an increasingly common condition that is characterized raised prevalence worldwide and associated with cardiovascular morbidity and mortality [1]. The results of few population-based and epidemiological investigations show that multiple risk factors and various metabolic comorbidities presented in CHF patients are able to affect nature evolution of cardiac failure [2-5]. Therefore, exiting differences in the prevalence of risk factors and comorbidities in patients with CHF may not completely explain sufficient distinguishes in survival in CHF patient population [6-8]. Recently, increasing attention has been paid to Insulin Resistance (IR) as a distinct cause of cardiac dysfunction and CHF in diabetic and non-diabetic patients [9,10]. IR mediates excessive or inadequate proliferation of the extracellular matrix accelerates apoptosis via increased oxidative stress, neurohumoral and inflammatory activation that negatively effect on cardiac remodeling, vasomotion, and endothelial function.

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Despite IR is considered a main component of metabolic syndrome and type two diabetes mellitus (T2DM), individuals with CHF may present IR prior to other dysmetabolic conditions [15,16]. However, the causality association of IR with none T2DM CHF patients is unclear and the underlying mechanisms of advance CHF affected IR have not been fully elucidated.

Recent studies have shown the role of circulating endothelial-derived microparticles (EMPs) in nature evolution of CHF with possibility predictive value [17-20]. Extracellular EMPs are defined as microvesicles with sizes ranging between 50 and 1000 nm released from plasma membrane of endothelial cells due to apoptosis or cell activation by specific (cytokine stimulation, mononuclear cooperation, coagulation, etc) and non-specific (shear stress) stimuli [21]. Apoptotic-derived or activated endothelium cell-derived EMPs are capable of transferring biological information, regulating peptides, hormones, proteins, lipid components without direct cell-to-cell contact to maintain cell homeostasis [22,23]. Interestingly, 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 [24,25]. In opposite, apoptotic-derived EMPs consist immune mediators, which are able to generating powerful signaling by the simultaneous receptor interaction and they are discussed a marker of endothelial cell injury and vascular aging [26,27].

However, the role of different immune phenotype in developing IR among CHF patients has not determined obviously. The aim of the study was to assess relationship between IR and immune phenotype of circulating EMPs in patients with CHF.

Methods

The study retrospectively involved 300 CHF patients aged 48 to 62 years who were undergone multispiral computed tomography angiography or coronary angiography in our centers between February 2011 and November 2013. Enrolled subjects presented atherosclerotic stenosis >50% of at least one coronary artery or they reported previously defined myocardial infarction. We excluded patients with acute infections; active inflammation; pulmonary edema; tachyarrhythmia; valvular heart disease; thyrotoxicosis; ischemic stroke; intracranial hemorrhage; surgery; trauma, autoimmune disease, malignancy, and acute coronary syndrome within 3 months prior to the study entry. All participants gave full written informed written consent.

Identification of immune phenotype of EMPs

Circulating EMPs were isolated from 5 ml of venous citrated blood drawn from the fistula-free arm. Platelet-free plasma (PFP) was separated from whole blood and then was centrifugated at 20,500×rpm for 30 min. EMPs pellets were washed with DMEM supplemented with 10 μg/ml polymyxin B, 100 U/ml streptomycin, and 100 U/ml penicillin and centrifuged again (20,500 rpm for 30 min). The obtained supernatant was extracted, and pellets were re-suspended into the remaining 200 μl of supernatant. PFP, EMPs, pellet, and supernatant were diluted five-, 10-, and five-fold in PBS, respectively [34].

Endothelial-derived apoptotic and activated microparticles were phenotyped by flow cytometry by phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (Platelet Endothelial Cell Adhesion Molecule [PECAM]-1), CD144 (Vascular Endothelial [VE]-cadherin), CD62E (E-selectin), and annexin V (BD Biosciences, USA) followed by incubation with fluorescein isothiocyanate (FITC)-conjugated annexin V (BD Biosciences, USA). Platelet-free plasma (PFP), EMPs, pellet, and supernatant were diluted five-, 10-, and five-fold in PBS, respectively [34].

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Statistical analysis

Statistical analysis of the results obtained was carried out in SPSS.
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Berezin AE (2015) Interrelationship between insulin resistance and impaired immune phenotype of circulating endothelial-derived microparticles in none-diabetic patients with chronic heart failure

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Left ventricular ejection fraction.

Density lipoprotein cholesterol, LDL-C: 
HbA1c: glycated hemoglobin, HDL-C: high-density lipoprotein cholesterol, BMI: body mass index, BNP: brain natriuretic peptide, LVEF: left ventricular ejection fraction.

Table 1. General characteristic of patients participating in study.

| Parameters                  | Entire cohort patients (n=300) | None IR subjects(n=129) | IR subjects(n=171) | P value |
|-----------------------------|-------------------------------|-------------------------|-------------------|---------|
| Age, years                  | 59.50 ± 7.30                 | 57.90 ± 8.10            | 60.30 ± 6.33      | 0.26    |
| Males, n (%)                | 186 (62.0%)                  | 77 (59.7%)              | 109 (63.7)        | 0.23    |
| Adherence to smoking, n (%) | 66 (22.0%)                   | 28 (21.7%)              | 38 (22.2%)        | 0.56    |
| Hypertension, n (%)         | 184 (61.3%)                  | 82 (63.6%)              | 102 (59.6%)       | 0.44    |
| NYHA class I, n (%)         | 76 (25.3%)                   | 34 (26.4%)              | 42 (24.5%)        | 0.62    |
| NYHA class II, n (%)        | 74 (24.7%)                   | 32 (24.8%)              | 42 (24.0%)        | 0.63    |
| NYHA class III, n (%)       | 98 (32.7%)                   | 45 (34.9%)              | 53 (31.0%)        | 0.60    |
| NYHA class IV, n (%)        | 52 (17.3%)                   | 18 (13.9%)              | 34 (19.9%)        | 0.12    |
| Dyslipidemia, n (%)         | 143 (47.7%)                  | 58 (45.0%)              | 85 (49.7%)        | 0.36    |
| Obesity, n (%)              | 122 (40.7%)                  | 44 (34.1%)              | 78 (45.6%)        | 0.042   |
| BMI, kg/m², M; 95% CI       | 24.2 (22.0-27.9)             | 23.07 (22.3-25.7)       | 25.99 (23.5-28.6) | 0.054   |
| GFR, mL/min/1.73 m², M; 95% CI | 85.2 (70.3-112.5)           | 82.8 (71.5-101.3)       | 87.4 (73.5-110.1) | 0.24    |
| HbA1c, %; M; 95% CI         | 5.8 (4.3-6.3)                | 5.5 (4.7-6.1)           | 6.1 (5.4-6.5)     | 0.012   |
| Fasting blood glucose, mmol/L, M; 95% CI | 5.10 (3.4-6.1)             | 4.97 (4.87-5.07)        | 5.47 (5.14-6.0)   | 0.001   |
| Insulin, µU/mL, M; 95% CI   | 13.12 (12.22-14.01)          | 10.41 (9.92-10.91)      | 15.15 (13.69-16.62) | 0.016   |
| Creatinine, µmol/L, M; 95% CI | 74.9 (65.1-90.3)            | 72.6 (69.31-88.1)       | 78.6 (70.2-89.1)  | 0.52    |
| Total cholesterol, mmol/L, M; 95% CI | 5.0 (4.2-5.8)             | 4.9 (4.1-5.3)           | 5.2 (4.5-5.7)     | 0.21    |
| LDL-C, mmol/L, M; 95% CI    | 3.02 (2.80-3.90)             | 3.00 (2.82-3.75)        | 3.11 (2.86-3.82)  | 0.044   |
| HDL-C, mmol/L, M; 95% CI    | 0.88 (0.82-0.97)             | 0.91 (0.86-0.95)        | 0.86 (0.83-0.92)  | 0.24    |
| NT-pro-BNP, pg/mL; M; 95% CI | 1533.6 (644.5-2560.6)       | 1066.9 (910.3-1223.6)   | 1480.5 (1310.4-1650.7) | 0.001   |
| hs-CRP, mg/L, M; 95% CI     | 7.34 (6.77-9.95)             | 7.16 (6.38-7.84)        | 7.51 (6.68-8.33)  | 0.016   |
| Systolic BP, mm Hg, M ± SD  | 129 ± 4                      | 131 ± 6                 | 129 ± 5           | 0.52    |
| Systolic BP, mm Hg, M ± SD  | 77 ± 5                      | 78 ± 4                  | 77 ± 6            | 0.48    |
| Heart rate, beats per 1 min, M ± SD | 76 ± 6                | 75 ± 4                  | 77 ± 6            | 0.54    |
| LVEF, %; M ± SD             | 46.07 ± 2.73                | 48.62 ± 1.64            | 44.15 ± 1.98      | 0.02    |

Note: Categorical variables are expressed as numbers (n) and percentages (%).

Abbreviations: M: mean value, CI: confidence interval, BP: blood pressure, NYHA: New York Heart Association, T2DM: type two diabetes mellitus, GFR: glomerular filtration rate, HbA1c: glycated hemoglobin, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, BMI: body mass index, BNP: brain natriuretic peptide, LVEF: left ventricular ejection fraction.

Results

General characteristic of the patients included in the study is reported in Table 1. Three hundred none diabetes patients with CHF (62.0% males) with mean age for 59.50 ± 7.30 years were divided into two cohorts depended on calculated value of HOMA-IR. Subjects with HOMA-IR >2.77 mmol/L × µU/mL were defined as patients with IR (n=171). However, patients with HOMA-IR <2.77 mmol/L × µU/mL were classified as none IR subjects (n=129). The patients of both cohorts were age- and sex-matched. Cardiovascular risk factors (adherence to smoking, hypertension, dyslipidemia) were found in both cohorts. However, obesity was appeared more frequent in IR patients. Therefore, IR patients had a significantly higher BMI, HbA1c, fasting blood glucose, insulin, hs-CRP, serum low-density lipoprotein cholesterol, NT-pro-BNP. Systolic and diastolic blood pressures, heart rate in both patient cohorts were comparable.

Concomitant medications in CHF patients included in the study are summarized in Table 2. Proportion of the patients included in both cohorts who were treated with ACE inhibitors or ARBs, mineralocorticoid receptor antagonists, loop diuretics, acetylsalicylic acid, and statins were similar. Beta-blockers and i/f blocker ivabradine were prescribed statistically much more in CHF subjects with IR when compared with none IR subjects (P=0.016). Other antiplatelet drugs were used more much frequently in none IR subjects (9.3%) than in IR patients (5.8%; P=0.046).

Immune phenotypes of EMPs in CHF patients were presented in Table 3. These were not significant differences between both cohorts patient in EMPs labeled as CD144+/CD31+, CD144+/annexin V+, and CD62E+ microparticles. In opposite, higher concentrations of CD144+/CD31+/annexin V+ EMPs and CD31+/annexin V+ EMPs were found in IR subjects when compared with none IR patients.

The univariate linear regression shown that numerous of CD144+/CD31+/annexin V+ EMPs was associated with NYHA class (r=0.59; P=0.001), HOMA-IR (r=0.46; P=0.001), NT-pro-BNP (r=0.42; P=0.001), LVEF (r=0.37; P=0.001), low-density lipoprotein cholesterol (r=0.32; P=0.001), hs-CRP (r=0.31; P=0.005), and TG (r =0.28, P=0.001). The numbers of CD31+/annexin V+ EMPs was directly

system for Windows, Version 20 (SPSS Inc, Chicago, IL, USA). The data were presented as mean (M) and standard deviation (±SD) or a 95% confidence interval (95% CI); the median (Me) and the 25%-75% interquartile range (IQR). The hypothesis of normal distribution of the parameters analyzed was checked by means of Shapiro–Wilk test and Kolmogorov-Smirnov test. To compare the main parameters of patients' groups (subject to the type of distribution of the parameters analyzed), one-tailed Student t-test or Shapiro–Wilk U-test were used. The two-tailed version of Wilcoxon test was used for paired comparison of parameter values inside the group. To compare categorical variables between groups, Chi² test (χ²) and Fisher F exact test were used. The factors, which could be associated potentially with circulating EMPs, were determined by means of univariate analysis of variance (ANOVA). Finally, we used logistic regression to calculate the odds ratio (OR) and a 95% CI for all the independent predictors of elevated circulating EMPs. The calculated difference of P<0.05 was considered statistically significant and all tests are reported two-tailed.
Interrelationship between insulin resistance and impaired immune phenotype of circulating endothelial-derived microparticles in none-diabetic patients with chronic heart failure

**Table 2. Concomitant medications in CHF patients included in the study.**

| Parameters                             | Entire cohort patients (n=300) | None IR subjects(n=129) | IR subjects (n=171) | P value |
|----------------------------------------|-------------------------------|------------------------|---------------------|---------|
| ACEI or ARAs, n (%)                    | 300 (100%)                    | 129 (100%)             | 171 (100%)          | 1.0     |
| Mineralcorticoid receptor antagonists, n (%) | 83 (27.7%)                 | 33 (25.6%)             | 50 (29.2%)          | 0.14    |
| Beta-blockers, n (%)                   | 237 (79.0%)                  | 88 (68.2%)             | 149 (87.1)          | 0.016   |
| Acetylsalicylic acid, n (%)            | 278 (92.7%)                  | 117 (90.7%)            | 161 (94.2%)         | 0.23    |
| Other antplatelet drugs, n (%)         | 22 (7.3%)                    | 12 (9.3%)              | 10 (5.8%)           | 0.046   |
| Ivabradine, n (%)                      | 89 (29.7%)                   | 26 (20.2%)             | 63 (36.8%)          | 0.026   |
| Loop diuretics, n (%)                  | 251 (83.7%)                  | 109 (84.5%)            | 142 (83.6%)         | 0.48    |
| Statins, n (%)                         | 143 (47.7%)                  | 58 (45.0%)             | 85 (49.7%)          | 0.36    |

**Abbreviations:** IR: insulin resistance

**Table 3. Immune phenotypes of EMPs in CHF patients.**

| Immune phenotypes | Entire cohort patients (n=300) | None IR subjects (n=129) | IR subjects (n=171) | P value |
|-------------------|-------------------------------|------------------------|---------------------|---------|
| CD144+/CD31+ EMPs, n/mL | 0.91 (0.36-1.35)             | 0.90 (0.34-1.27)       | 0.93 (0.41-1.32)    | 0.62    |
| CD144+/annexin V+ EMPs, n/mL | 1.15 (0.13-2.41)             | 1.13 (0.18-2.22)       | 1.17 (0.16-2.35)    | 0.24    |
| CD144+/CD31+annexin V+ EMPs, n/mL | 1.01 (0.39-1.70)             | 0.98 (0.35-1.53)       | 1.03 (0.44-1.63)    | 0.044   |
| CD31+/annexin V+ EMPs, n/mL | 0.296 (0.261-0.339)          | 0.278 (0.243-0.310)    | 0.315 (0.289-0.327) | 0.001   |
| CD62E+ EMPs, n/mL | 1.03 (0.86-1.13)             | 1.05 (0.94-1.11)       | 1.02 (0.81-1.10)    | 0.73    |

**Note:** The values are presented as the median and 25-75% interquartile range, the differences validity values obtained by two-tailed Mann-Whitney test.

**Abbreviations:** IR: insulin resistance, EMPs: endothelial-derived microparticles.

**Table 4. C-statistics for Models with HOMA-IR, NYHA class, hs-CRP, NT-proBNP as Continuous Variables.**

| Models                                              | Dependent variable: CD144+/CD31+ annexein V+ EMPs | Dependent variable: CD31+/annexin V+ EMPs |
|-----------------------------------------------------|----------------------------------------------------|------------------------------------------|
|                                                     | AUC (95% CI) | ΔAUC | IDI (±SE) | Relative IDI (%) | AUC (95% CI) | ΔAUC | IDI (±SE) | Relative IDI (%) |
| Model 1 (based model: HOMA-IR=2.77mmol/L·µU/mL)     | 0.669       | -    | -         | -               | 0.664       | -    | -         | -               |
| Model 1 + NYHA class + biomarkers (hs-CRP, NT-proBNP) | 0.681       | -    | -         | -               | 0.685       | -    | -         | -               |
| Model 1 + NYHA class + biomarkers (hs-CRP, NT-proBNP) versus Model 1 | 0.012; P=0.64 | 0.02 ± 0.015 | 1.8% | - | 0.021; P=0.12 | 0.03 ± 0.012 | 2.2% |

**Note:** Relative IDI: calculated as the ratio of IDI over the discrimination slope of the model without IR.

**Abbreviations:** AUC: area under curve, SE: standard error, IR: insulin resistance, BNP: brain natriuretic peptide, hs-CRP: high sensitive C-reactive protein.

**Discussion**

The results of our investigations shown that IR in none-diabetic population of CHF patients may consider a predictor of impaired phenotype of circulating EMPs, which reflects surplus of apoptotic-derived microparticles in circulation association with probably relatively deficiency of activated endothelial cell-derived.
Berezin AE (2015) Interrelationship between insulin resistance and impaired immune phenotype of circulating endothelial-derived microparticles in none-diabetic patients with chronic heart failure

Limitations of the study

This study has some restrictions. First our study is limited by its retrospective nature and small sample size. The authors believe that a greater cohort is to be desirable to improve the power of the study. Therefore, there were several technical-related difficulties in the measurement of EMPs. In fact, lack of standard protocol for isolating and detecting circulating EMPs obtained from the plasma. According opinion of the majority experts, centrifugation is became the main and detecting circulating EMPs obtained from the plasma. According to the measurement of EMPs. In fact, lack of standard protocol for isolating and detecting circulating EMPs obtained from the plasma.

We found that IR remained a statistically significant predictor for increased apoptotic-derived EMPs that are able to mediate endothelial inflammation and decrease ability to endothelial repair. Thus, impaired apoptotic phenotype in CHF patients reflects a limiting capacity of endothelial cell to maintain cardiac function in the face of co-morbidities such as IR. Whether these findings are predictable for non-diabetic patients with CHF is not obviously understood. Moreover, it is not clear whether serial measurements of circulating EMPs are considered a diagnostic tool for risk stratification of the patients with CHF. More studies are underway to evaluate the role of IR in impaired phenotype of circulating EMPs among CHF subjects.

Conclusion

We found that IR remained a statistically significant predictor for increased apoptotic-derived EMPs labelled as CD144+/CD31+/annexin V+ and CD31+/annexin V+ EMPs in none-diabetic patients 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, and wrote the manuscript. Alexander A. Kremzer contributed to enroll the patients, collected and analyzed the data, checked clinical events and reviewed the source documents. Yulia V. Martovitskaya contributed circulating biomarker determination, preformed preparation of isolates of microparticles in samples with further phenotyping by flowcytometry, and interpreted the obtained results. Tatyana A. Samura preformed visualization procedures and interpreted the obtained results. Tatyana A. Berezina contributed in biological variability of EMP count. Although HD-FACS methodology is widely used, theoretically overlap between two or more fluorochromes might reflect some obstacles for further interpretation of obtained results. Another limitation of the present study is that a specific role of EMPs is also possible and has not been characterized in depth in T2DM patients. However, the authors suppose that these restrictions might have no significant impact on the study data interpretation. Additionally, retrospective, relative small sample size may limit the significance of the present study. However, this was not a randomized and controlled study. The authors believe that a greater cohort of patients with more incidences detected is desirable to prove the credibility of the study.

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Ethical principles

All the patients have given their written informed consent for participation in the study. The investigators followed strictly all the requirements to clinical trials in conformity with the World Medical Association Declaration of Helsinki, 1964, Good Clinical Practice provided by International Conference on Harmonization, Council of Europe Conventionfor theProtectionof Human Rights and Dignityofthe Human Being in view of using achievements in biology and medicine, Convention on Human Rights and Biomedicine, including Additional Protocol to the Conventionon Human Rights and Biomedicine,concerningBiomedical Research, and legislation of Ukraine.

Authors' contributions

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

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