Research Paper

Pattern of endothelial progenitor cells and apoptotic endothelial cell-derived microparticles in chronic heart failure patients with preserved and reduced left ventricular ejection fraction

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ABSTRACT

Background: Chronic heart failure (HF) remains a leading cause of cardiovascular (CV) mortality and morbidity worldwide. The aim of the study was to investigate whether the pattern of angiogenic endothelial progenitor cells (EPCs) and apoptotic endothelial cell-derived microparticles (EMPs) would be able to differentiate HF with reduced (HFrEF) and preserved (HFpEF) ejection fraction.

Methods: One hundred sixty four chronic HF subjects met inclusion criteria. Patients with global left ventricular ejection fraction ≥50% were categorized as the HFpEF group (n = 79) and those with ≤45% as the HFrEF group (n = 85). Therefore, to compare the circulating levels of biological markers 35 control subjects without HF were included in the study. All control individuals were age- and sex-matched chronic HF patients. The serum level of biomarkers was measured at baseline. The flow cytometric technique was used for predictably distinguishing circulating cell subsets depending on expression of CD45, CD34, CD14, Tie-2, and CD309 antigens and determining endothelial cell-derived microparticles. CD31$^+$/annexin V$^+$ was defined as apoptotic endothelial cell-derived MPs, MPs labeled for CD105$^+$ or CD62E$^+$ were determined as MPs produced due to activation of endothelial cells.

Results: In multivariate logistic regression model T2DM (R^2 = 0.26; P = 0.001), obesity (R^2 = 0.22; P = 0.001), previous MI (R^2 = 0.17; P = 0.012), galectin-3 (R^2 = 0.67; P = 0.012), CD31$^+$/annexin V$^+$ EMPs (R^2 = 0.11; P = 0.001), NT-proBNP (R^2 = 0.11; P = 0.046), CD14$^+$CD309$^+$ cells (R^2 = 0.058; P = 0.001), and CD14$^+$CD309$^+$ Tie-2$^+$ cells (R^2 = 0.044; P = 0.028) were found as independent predictors of HFpEF. Using multivariate Cox-regression analysis adjusted etiology (previous myocardial infarction), cardiovascular risk factors (obesity, type 2 diabetes mellitus) we found that NT-proBNP (OR 1.08; 95% CI = 1.03–1.12; P = 0.001) and CD31$^+$/annexin V$^+$ EMPs to CD14$^+$CD309$^+$ cell ratio (OR 1.06; 95% CI = 1.02–1.11; P = 0.02) were independent predictors for HFpEF.

Conclusion: We found that CD31$^+$/annexin V$^+$ EMPs to CD14$^+$CD309$^+$ cell ratio added to NT-proBNP, clinical data, and cardiovascular risk factors has exhibited the best discriminate value and higher reliability to predict HFpEF compared with NT-proBNP and clinical data/cardiovascular risk factors alone.

1. Introduction

Chronic heart failure (HF) remains a leading cause of cardiovascular (CV) mortality and morbidity worldwide (Go et al., 2014). Although over the last decades the incidence of newly HF in developed countries have been substantially declined particularly for HF with reduced ejection fraction (HFrEF) (Gerber et al., 2015), there is marked increase in hospital admissions, CV and non-CV death rate predominance of HF with preserved ejection fraction (HFpEF) (Dunlay et al., 2015; Jorge et al., 2015). As expected, the routine use of biomarkers on diagnosis of HFrEF and HFpEF might help stratify the patients at higher risk of death and clinical outcomes. In fact, both 2012 European Society of Cardiology (ESC) Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure and 2013 American College of Cardiology Foundation/ American Heart Association (ACCF/AHA) Guideline for the Management

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of Heart Failure are well accepted by many clinicians regarding HFrEF diagnosis. Indeed, the HFpEF is that one that really needs improvement of biomarkers for diagnosis and prognosis (McMurray et al., 2012; Yancy et al., 2013). In this context, many biological markers, which reflect several faces of pathogenesis of HF, have been investigated in detail, but by now natriuretic peptides, soluble ST2, galectin-3, and high-sensitive cardiac specific troponins were validated only. However, there was not a large body of evidence regarding perspectives that may provide clinically useful prognostic information both concerning the future risk of HFpEF/HFrEF manifestation in asymptomatic subjects, the risk of fatal events and primary/re-admissions in the hospital in individuals for those have already established symptomatic acute, acutely decompensated/advanced, and chronic stable HF related to ischemic and non-ischemic causes (D'Elia et al., 2015). It is suggested that multimorbidity in HF may limit the diagnostic and predictive utility of biomarkers (Chamberlain et al., 2015).

Recent studies showed that endothelium injury is common for HF onset and development beyond etiology (Fujisue et al., 2015). Endothelial dysfunction closely associates with activation and/or apoptosis of endothelial cells lead to release of newly detectable circulating biomarkers related to endothelial dysfunction called endothelial cell-derived microparticles (EMPs) (Dignat-George and Boulanger, 2011; Burger and Touyz, 2012). Human $CD34^+$ primitive progenitors and $CD14^+$ monocytic progenitors have exhibited pro-angiogenic capacities mediated through increased sensitivity to vascular endothelial growth factor and cell-to-cell cooperation via secretion of endothelial cell-derived microparticles (Awad et al., 2006; Burger and Touyz, 2012).

Therefore, endothelial progenitor cells (EPCs) labeled as $CD14^+$-$CD309^+$(VEGFR2) and $CD14^+ CD309^+$(VEGFR2) Tie-2 + cells were found a marker of endothelial dysfunction and reparation ability (Dignat-George and Boulanger, 2011). It has been suggested that imbalance between EPCs with angiogenic capacity and apoptotic EMPs contributed in cell injury and endothelial dysfunction may reflect impaired reparative phenotype that is suitable for several CV diseases including HF (Berezin, 2015a; Berezin and Kremzer, 2015a,b; Berezin et al., 2015a). Indeed, endogenous deficiency of angiopoetic stimuli mediated by secretion of pro-inflammatory cytokines, neurohormones, growth factors, might lead to worsening endothelium reparation and HF progression (Singh et al., 2012; Berezin et al., 2015b). Recently we have reported that apoptotic EMP to EPC ratio might independently predict clinical outcomes in advanced chronic HFrEF patients (Berezin et al., 2015c). However, whether impaired reparative phenotype might reflect a development of HFpEF and HFpEF is still not clear. The aim of the study was to investigate whether the pattern of endothelial progenitor cells with angiogenic capacity and apoptotic endothelial cell-derived microparticles would be able to associate with HFpEF and HFpEF phenotypes.

2. Methods

A total of 228 subjects suspected chronic HF were selected in this study after reviewing discharge reports. All these persons were treated in Zaporozhye Regional Hospital, City Hospital #6, City Hospital #10, Zaporozhye Regional Center of Cardiovascular Diseases from April 2010 to June 2015 with primary diagnosis chronic HF.

Chronic HF was defined according to contemporary criteria provided by actual clinical recommendation (McMurray et al., 2012). HFrEF (LVEF ≤ 45%) and HFpEF (LVEF ≥ 50%) were determined accordingly this recommendation. T2DM was diagnosed with revised criteria provided by American Diabetes Association when source documents were reviewed (Executive summary, 2013). When one or more of the following components were found (glycated hemoglobin [HbA1c] ≥6.5%; fasting plasma glucose ≥7 mmol/L; 2-h plasma glucose ≥11.1 mmol/L during an oral glucose tolerance test; a random plasma glucose ≥11.1 mmol/L; exposure of insulin or oral anti-diabetic drugs; a previous diagnosis of T2DM) T2DM was determined. Dyslipidemia was checked and determined according to NCEP Adult Treatment Panel

III (National Cholesterol Education Program) (National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2002).

Including criteria for selection of the HF patients in the study were LVEF <59%, ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (E') [E/E'] ratio > 15 units, elevated level of serum NT-proBNP >220 pg/mL, and clinical presentation of chronic HF. Excluding criteria were severe kidney and liver diseases; malignancy; creatinine plasma level above 440 μmol/L; estimated GFR index <35 mL/min/m²; brain injury within 3 months before the enrollment; valvular heart disease; thyrotoxicosis; ischemic stroke; intracranial hemorrhage; acute infections; surgery; trauma; pregnancy; implanted pacemaker/defibrillator/cardioverter.

The flow chart representing patient in the study is reported in Fig. 1.

Among these 228 prescreened subjects, only 164 chronic HF subjects were included in the study accordingly inclusion/exclusion criteria. Patients with global left ventricular ejection fraction >55% were categorized as the HFpEF group (n = 79) and those with ≤45% as the HFrEF group (n = 85). Therefore, to compare the circulating levels of biological markers 35 control subjects without HF were included in the study. To compare EPCs and microparticles between healthy subjects, HFpEF and HFrEF individuals control group was made. Control subjects are defined as individuals with normal global cardiac function (LVEF >55%, E/E' ratio < 8 units) assessed by transthoracic echocardiography and Tissue Doppler Imaging, serum NT-proBNP level <125 pg/mL, and without any signs and symptoms of symptomatic HF (Fig. 2).

2.1. Ethical Statement

The study protocol was approved by the local Ethics Committee Review Board (IRB # 3/2010), State Medical University of Zaporozhye (Ukraine) prior to the study initiation. The study complied with the Declaration of Helsinki and voluntary informed written consent was obtained from all patients included in this study. All individuals included in the study have given voluntary informed written consent.

2.2. Anthropometric Measurements

Anthropometric measurements were made using standard procedures.

2.3. Echocardiography and Doppler Imaging

Transthoracic B-mode echocardiography and Tissue Doppler Imaging were performed according to a conventional procedure on ACUSON scanner (Siemens, Germany) using phased probe with modulated frequency of 2.5–5 MHz. Left ventricular end-diastolic and end-systolic volumes, and LVEF were measured by modified Simpson's method (Quiñones et al., 2003). E/E' ratio was measured using pulsed wave Tissue Doppler Imaging according contemporary protocol (Paulus et al., 2007).

2.4. Glomerular Filtration Rate Measurement

Calculation of glomerular filtration rate (GFR) was calculated by CKD-EPI formula (Levey et al., 2009).

2.5. Blood Sampling

After an overnight fast blood samples were drawn 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 immediately centrifuged upon permanent cooling at 6000 rpm for 10 min. Then, plasma was refrigerated immediately to be stored at a temperature − 70 °C. All laboratory tests were performed using standard methods to measure the serum fasting plasma glucose, fasting lipid profiles and other biomarkers.