BBA Clinical 3 (2015) 18–24

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: http://www.journals.elsevier.com/bba-clinical/

The predictive role of circulating microparticles in patients with chronic heart failure ☆

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

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

ARTICLE INFO

Article history:
Received 7 November 2014
Received in revised form 25 November 2014
Accepted 29 November 2014
Available online 5 December 2014

Keywords:
Chronic heart failure
Microparticles
Predictive value
Cardiovascular outcomes

ABSTRACT

Aim: The study aim was to evaluate whether circulating microparticles with apoptotic or non-apoptotic phenotypes are useful for risk assessment of 3-year cumulative fatal and non-fatal cardiovascular events in CHF patients.

Methods: The incidence of fatal and non-fatal cardiovascular events, as well as the frequency of occurrence of death from any cause in a cohort of 388 patients with CHF during 3 years of observation was studied prospectively. Circulating levels of NT-pro brain natriuretic peptide (NT-pro-BNP), high-sensitivity C-reactive protein (hs-CRP), and endothelial apoptotic microparticles (EMPs) were measured at baseline.

Results: Median follow-up was 2.32 years (IQR = 1.8–3.1). During follow-up, 110 cardiovascular events (including 43 fatal cases) were determined. Additionally, 74 subjects were hospitalized repetitively due to worsening CHF and also 16 subjects were readmitted in the hospital due to other cardiovascular reasons. In the univariate logistic regression analysis, the main factors independently related with cumulative endpoints were creatinine, fasting glucose, HbA1c, total cholesterol, uric acid, various types of EPMs, NT-pro-BNP, hs-CRP, NYHA class, decreased left ventricular ejection fraction (LVEF) less 45%, and type 2 diabetes mellitus. In multivariate model NYHA class, decreased LVEF (less 45%), NT-pro-BNP, hs-CRP, CD144 +/CD31 +/annexin V + EMPs, and CD31 +/annexin V + EMPs remained statistically significant for cumulative endpoint. Adding of CD144 +/CD31 +/annexin V + EMCs and CD31 +/annexin V + EMCs to the standard ABC model may improve the relative IDI for cumulative endpoint by 11.4% and 10.5% respectively.

Conclusion: Apoptotic phenotype of circulating microparticles may relate 3-year combined clinical outcomes in CHF patients.

1. Introduction

Chronic heart failure (CHF) remains a leading cause of cardiovascular morbidity and mortality [1]. Moreover, the frequencies of novel cases of CHF arise progressively worldwide [2]. Although the endothelium is considered an important target for traditional risk factors and endothelial dysfunction remained independently associated with mortality from CHF [3,4], the innate molecular mechanisms affected forming endothelial dysfunction are not fully clear. In this context, systemic pro-inflammatory activation, metabolic comorbidities, and neurohumoral state are considered the origin of microvascular endothelial cell inflammation that leads to the development of CHF and supports cardiac remodeling and vascular dysfunction [4]. Moreover, endothelial dysfunction is suggested as an early event in the development and progression of CHF. However, biological markers of endothelial dysfunction are abundant; there remains no ideal indicators that relate to the several faces of the pathogenesis of CHF [5,6]. The European Society of Cardiology and the American Heart Association/American Colleges of Cardiology have recently published a set of current biomarkers with high predictive value and powerful diagnostic capacity for subjects with CHF, which include natriuretic peptides, cardiac specific troponin, galectin-3, and high-sensitivity C-reactive protein [7–9]. Although all these markers have obvious advantages and some disadvantages too, there are several limitations regarding interpretations and implementation of obtained findings in risk stratification among CHF patients [10]. Collectively, discovering new biomarkers that closely

☆ This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

* Corresponding author. Tel.: +380 612894585.
E-mail address: dr_berezin@mail.ru (A.E. Berezin).

http://dx.doi.org/10.1016/j.bbacli.2014.11.006

related with nature evolution of CHF and tightly reflected different stages of disease appears to be attractive. Taking into consideration the pathogenesis of CHF and the role of endothelial dysfunction in nature evolution of cardiac failure, small-size cell membrane vesicles, such as microparticles, that are derived from activated cells or apoptotic particles, might be useful for risk stratification of the subjects with CHF. Indeed, wide spectrum endothelial cell-, platelet-, and monocyte/macrophage-derived microparticles have recently been associated with cardiovascular and metabolic diseases, inflammatory state, and autoimmune conditions.

Endothelial-derived microparticles (EMPs) are a novel biological marker of endothelial injury and vascular tone disorders [11,12]. EMPs are defined as a heterogeneous population of vesicles (diameter of 100–1000 nm) that are released by cellular vesiculation and fission of the membrane of endothelial cells [13]. EMPs derive from activated or apoptotic endothelial cells and may play a pivotal role in the vascular remodeling and endothelial reparation [14,15]. Biological effect of EMPs may mediate by supporting cell-to-cell cross-talking because EMPs transport miRNA, active molecules, hormones, peptides, regulator proteins, etc. [16,17]. Probably, EMPs may contribute to hyperadrenergic state in CHF via regulation of adrenal signaling [18,19]. However, knowledge on EMPs is sufficiently limited due to their submicrometer size and to intrinsic limitations in methods applied for their determination, while their role in CHF is probably underestimated. The study aim was to evaluate whether circulating endothelial-derived microparticles with apoptotic or non-apoptotic phenotypes are useful for risk assessment of 3-year cumulative fatal and non-fatal cardiovascular events in CHF patients.

2. Methods

2.1. Study population

The study population consisted of 388 consecutive patients with CHF who underwent angiography or PCI between April 2010 and June 2014, as well as referred as post-myocardial infarction subjects within this period in the five centers which participated in this investigation. All these patients were selected from 1427 patients according to our inclusion and exclusion criteria. The study protocol was approved by the Zaporozhye State Medical University ethics committee review board. The study complied with the Declaration of Helsinki and voluntary informed written consent was obtained from all patients included in this study.

Prognosis was assessed by the composite endpoint related to all-cause death, CHF-related death or CHF hospitalization, censored at 3 years.

2.2. Methods for visualization of coronary arteries

Multispiral computed tomography angiography and/or angiographic study have been carried out to verify the ischemic nature of the disease in patients. Multispiral computed tomography angiography has been carried out for all the patients prior to their inclusion in the study. When atherosclerotic lesions of the coronary arteries were verified, patients were subjected to conventional angiographic examination provided indications for revascularization were available. CAD was considered to be diagnosed upon availability of previous angiographic examinations carried out not later than 6 months ago provided no new cardiovascular events occurred for this period, and the procedure is available for assay. The coronary artery wall structure was measured by contrast-enhanced spiral computed tomography angiography [20] on Somatom Volume Zoom scanner (Siemens, Erlangen, Germany) with two detector rows using non-ionic contrast Omnipaque (Amersham Health, Ireland).

2.3. Echocardiography and tissue Doppler imaging

Transthoracic B-mode echocardiography and tissue Doppler imaging were performed according to a conventional procedure on ACUSON scanner (SIEMENS, Germany) using phased transducer of 5 MHz. Left ventricular end-diastolic and end-systolic volumes, and ejection fraction (LVEF) were measured by modified Simpson's planimetric method [21,22]. Inter- and intra-observer variability coefficients for LVEF were 3.2% and 1.1% respectively.

2.4. Glomerular filtration rate measurement

Calculation of glomerular filtration rate (GFR) was calculated by CKD-EPI formula [23].

2.5. Biomarker determination

All biomarkers were determined at baseline. To measurement of biological marker concentrations, blood samples were drawn in the morning (at 7–8 a.m.) into cooled silicone test tubes. Samples were processed according to the recommendations of the manufacturer of the analytical technique used. They were centrifuged upon permanent cooling at 6000 rpm for 3 min. Then, plasma was refrigerated immediately to be stored at a temperature −70 °C until measurement.

Circulating NT-pro-BNP level was measured by immunoelectrochemoluminescent assay using sets produced by R&D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). The high-sensitivity C-reactive protein (hs-CRP) levels were measured by using nephelometric technique on AU640 analyzer manufactured by Diagnostic Systems Group (Japan).

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

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

2.6. Endothelial-derived apoptotic and activated microparticle determination

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) per HD-FACS (High-Definition Fluorescence Activated Cell Sorter) methodology. The samples were incubated in the dark for 15 min at room temperature according to the manufacturer's instructions. The samples were then analyzed on a FC500 flow cytometer (Beckman Coulter). For determination of annexin V+ EMPs 400 μL annexin V binding buffer was added. For each sample, 500,000 events have been analyzed. EMPs gate was defined by size, using 0.8 and 1.1 mm beads (Sigma, St. Louis, MO, USA). CD31+/annexin V+ and CD144+/CD31+/annexin V+ microparticles were defined as apoptotic EMPs, EMPs positively labeled for CD62E+ were determined as EMPs produced due to activation of endothelial cells. Therefore, double-positive EMPs (CD31 and CD144) and triple-positive (CD144+/CD31+/annexin V+) were defined as most specific EMPs [24,25].

2.7. Statistical analysis

Statistical analysis of the results obtained was carried out in SPSS system for Windows, Version 22 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism for Windows, Version 5 (GraphPad Software Inc., La Jolla, CA, USA). The data were presented as mean (M) and standard