Diabetes & Metabolic Syndrome: Clinical Research & Reviews 10 (2016) 29–36

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original article

Pattern of circulating endothelial-derived microparticles among chronic heart failure patients with dysmetabolic comorbidities: The impact of subclinical hypothyroidism☆

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

[a] Cardiology Unit, 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

Keywords:
Chronic heart failure
Dysmetabolic comorbidities
Subclinical hypothyroidism
Endothelial-derived microparticles

SUMMARY

The study aim was to evaluate the impact of dysmetabolic comorbidities including subclinical hypothyroidism (SH) on pattern of circulating endothelial-derived microparticles (EMPs) in chronic heart failure (CHF) patients.

Methods: It was retrospectively involved a cohort of 388 patients with CHF. Fifty three CHF subjects had SH and 335 patients were free from thyroid dysfunction. Circulating levels of NT-pro brain natriuretic peptide (NT-pro-BNP), high-sensitivity C-reactive protein (hs-CRP), thyroid stimulating hormone (TSH), total and free thyroxine (T4) and triiodothyronine (T3) EMPs were measured at baseline. SH was defined per contemporary clinical guideline as state associated with elevated level of serum TSH > 10 μU/L and basal normal free T3 and T4 concentration.

Results: Circulating CD31+/annexin V+ EMPs were higher in SH patients compared with none SH subjects. In contrast, activated CD62E+ EMP numbers were not significantly different between both patient cohorts. Using C-statistics for Models with TSH, New York Heart Association (NYHA) class, dyslipidemia, and circulating biomarkers (hs-CRP, NT-proBNP, serum uric acid) as Continuous Variables we found that adding of NYHA class alone, NT-proBNP alone or their combination to the based model (TSH) improved the relative integrated discrimination improvement (IDI) for increased CD31+/annexin V+ to CD62E+ ratio by 4.9%; 9.2% and 9.6% respectively. NT-proBNP improves significantly predictive model based on TSH for increased CD31+/annexin V+ to CD62E+ ratio. Among patient study population for category-free net reclassification improvement (NRI), 4% of events ($P = 0.026$) and 6% of non-events ($P = 0.012$) were correctly reclassified by the addition of circulating NT-proBNP to the base model (TSH) for Increased CD31+/annexin V+ to CD62E+ ratio. Therefore, 4% of events ($P = 0.028$) and 5% of non-events ($P = 0.014$) were correctly reclassified using category-free NRI for increased CD31+/annexin V+ to CD62E+ ratio.

Conclusion: We found that SH state in CHF patients associates with impaired pattern of circulating EMPs with predominantly increased number of apoptotic-derived microparticles.

1. Introduction

Subclinical hypothyroidism (SH) is a biochemical diagnosis defined as a normal serum free thyroxine (T4) concentration in the presence of an elevated serum thyroid-stimulating hormone (TSH) level [13]. Hypothyroidism among non-pregnant female and adult male has multiple etiologies and frequently associates with cardiovascular (CV) diseases and CV risk factors [16,17]. Indeed, the recent studies have emerged a strong independent association between SH and atherosclerosis [29], carotid plaque ulceration

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

Abbreviations: BMI, body mass index; BMP, brain natriuretic peptide; CI, confidence interval; CHF, chronic heart failure; EMPs, endothelial-derived microparticles; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; OR, odds ratio.

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

http://dx.doi.org/10.1016/j.dsx.2015.08.001
1871-4021/

[34], cerebrovascular events [34], chronic heart failure (CHF) [44]. Therefore, SH frequently accompanies with dyslipidemia, overweigh/obesity, oxidative stress, low intensity inflammation, coagulation, and may discuss a population attributable risk factor [15]. Moreover, SH may directly accelerate endothelial dysfunction through specific molecular pathways in endothelial cells affected NO production and increase of degradation of vasodepressor intermediates [18]. Interestingly, the role of SH in cardiovascular morbidity and mortality is controversial and is active investigated [42]. Because SH is relatively common in older and seniors patients, conflicting results on the age-related association between SH and CV risk and CV events have been found [32,37]. Mashburn and Whiteley [44] reported that SH in elderly patients was not associated with increased risk for coronary heart disease, stroke, peripheral arterial disease, or cardiovascular-related or total mortality. Although total mortality was not increased among SH subjects, severity of SH that is graduated as attribute of elevated serum TSH level closely associates with CV outcomes and mortality in unselected adult patient population [2,14,31]. Overall, SH may contribute CV risk and disease development via endothelial dysfunction. In this context, circulating endothelial-derived microparticles (EMPs) as novel biological markers of endothelial injury, vascular tone disorders, and vascular aging [2,30] may display an impact of SH in CV disease progression.

EMPs are defined a heterogeneous population of vesicles (diameter 100–1000 nm) that are released by cellular vesiculation and fission of the membrane of endothelial cells [25]. Biological effect of EMPs may mediate through supporting of cell-to-cell cross-talking because EMPs transport miRNA, active molecules, hormones, peptides, regulator proteins, etc. [24]. EMPs derive from activated or apoptotic endothelial cells and may play a pivotal role in the endothelial reparation, tissue injury, and vascular remodeling [10]. The different pattern of circulating EMPs in CV diseases including CHF suggests being impaired phenotype of EMPs that is probably available for the risk stratification among CV and metabolic disease subjects [4,11, 27,46]. However, the causality role of EMP pattern in CHF patients with SH is still unclear. The study aim was to evaluate the impact of dysmetabolic comorbidities including SH on pattern of circulating EMPs in CHF patients.

2. Methods

2.1. Study population

The study retrospectively involved a cohort of 388 patients with documented CHF who underwent angiography or percutant coronary artery intervention (PCI) between April 2010 and June 2014 or they were referred as post-myocardial infarction subjects. Fifty three CHF subjects had SH and 335 patients were free from thyroid dysfunction.

The study protocol was approved by the Zaporozhye State medical University Ethnics committee review board (#3 12/02/2012). The study complied with the Declaration of Helsinki and voluntary informed written consent was obtained from all patients included in this study.

2.2. Methods for visualization of coronary arteries

Multispiral contrast-enhanced computed tomography angiography or conventional angiography was performed for all the patients prior to their inclusion in the study. The coronary artery wall structure was measured by contrast-enhanced spiral computed tomography angiography on Somatom Volume Zoom scanner (Siemens, Erlangen, Germany) [5] 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 probe of 2.5–5 MHz. Left ventricular end-diastolic and end-systolic volumes, and ejection fraction (LVEF) were measured by modified Simpson's method [33]. 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 [20].

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 brain natriuretic peptide (NT-pro-BNP), high-sensitivity C-reactive protein (hs-CRP), TSH, total and free thyroxine (T4), total and free triiodothyronine (T3) were measured by using immune electrochemiluminescence 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 enzymatic method. Concentration of cholesterol of low-density lipoproteins (LDL-C) was calculated according to the Friedewald formula (1972) [12].

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

2.6. Determination of subclinical hypothyroidism

SH was defined per contemporary clinical guideline as state associated with elevated level of serum TSH > 10 μU/L and basal normal free T3 and T4 concentrations [35].

2.7. Endothelial-derived apoptotic and activated microparticles 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 high-definition fluorescence activated cell sorter (HD-FACS) 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 thousand 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