Circulating Endothelial Progenitor Cells as Markers for Severity of Ischemic Chronic Heart Failure

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ABSTRACT

Introduction: Despite a high potential of endothelial progenitor cells (EPCs) for diagnostic purposes, the EPC role in developing ischemic chronic heart failure (CHF) has not been determined obviously.
The objective of this study was to assess the counts of $CD45^+CD34^+$, $CD45^-CD34^+$, $CD14^+CD309^+$, and $CD14^+CD309^+Tie2^+$ phenotyped circulating EPCs of various subpopulations in patients with ischemic CHF.

Methods and Results: The study involved 153 patients (86 male), aged 48−62 years, with angiographically proven coronary artery disease (CAD) and 25 healthy volunteers. CHF was diagnosed in 109 patients (71.2%). Mononuclear cell populations were phenotyped by flow cytofluorimetry. Cardiovascular risk factors, such as type 2 diabetes mellitus, hyperlipidemia, arterial hypertension, and adherence to smoking, may have a negative effect on circulating EPC counts in CAD patients regardless of the presence of CHF. The depletion of the $CD14^+CD309^+$- and $CD14^+CD309^+Tie2^+$-phenotyped circulating EPC counts is associated with the severity of left ventricular dysfunction, whereas the $CD45^+CD34^+$- and $CD45^-CD34^+$-mononuclear cell counts are more representative of the severity of atherosclerotic coronary artery lesions.

Conclusion: The authors found that New York Heart Association functional class of CHF, left ventricular ejection fraction $<42\%$, the N-terminal pro−B-type natriuretic peptide level >554 pg/mL, and E/Em ratio >15 U had the highest predictive value for the depletion of the EPC count in CAD patients. (*J Cardiac Fail 2014;20:438−447*)

Key Words: Coronary artery disease, mononuclears, predictive value.

A number of earlier studies have shown the role of circulating endothelial progenitor cells (EPCs) of hematopoietic origin in the pathogenesis of cardiovascular diseases.[1,2] For example, it has been found that the $CD34^+CD45^-$-phenotyped EPC count may increase in patients with myocardial infarction, unstable angina pectoris, or acute coronary syndrome,[3,4] and may decrease in patients with subclinical atherosclerosis, chronic heart failure (CHF), or total contractile myocardial dysfunction.[5,6] Circulating EPCs ensure reparative processes, including endothelization of the fragments of vascular lesion, as well as remodeling of extracellular matrix and neoangiogenesis.[7,8] Mobilization of endothelial progenitor cells, which possess a potential for angiopoiesis and organ protection, is regulated by a wide range of proinflammatory cytokines, signal molecules, including micro-RNA, ischemia-induced factors, neurohormones, glycopeptides, and products of oxidative stress.[3] Mature endothelial cells also may differentiate from activated mononuclear cells mobilized from peripheral tissues.[9,10] Circulating EPCs of nonhematopoietic origin, which express $CD34^+CD45^-$, are phenotypically identical with primitive progenitor cells of other origin; they are functionally different from primitive progenitor cells of other origin only in their colony-formation ability when cultivated.[11,12] This raises difficulties when identifying the EPC production source if expression of CD34 antigen

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See page 446 for disclosure information.

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in $CD45^-$ mononuclear cells was verified. Moreover, no association has been verified between the $CD34^+CD45^-$-phenotype circulating cells count on the one hand and the severity of coronary atherosclerosis and patients' survival rate on the other hand.[13] That has driven attempts to verify other subpopulations of EPCs coexpressing CD34 antigen and vascular endothelial growth factor receptor (VEGFR) 2 vascular growth ligands, CD133, CD14, and tyrosine kinase ligand (Tie2). $CD34^+$ granulocytes, which express Tie2 and VEGFR2, are supposed to include activated mononuclear cells of nonhematopoietic origin, which have phenotypic differences manifested in additional expression of CD14 antigen, show their pluripotency, and are a potential source of endotheliocytes.[14] For EPCs of $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ phenotypes, association has been found with atherosclerosis incidence and survival of patients with acute coronary syndrome and myocardial infarction.[15] However, despite significant steps forward in defining EPC potential for diagnostic purposes, the role of EPCs of hematopoietic and nonhematopoietic origin in developing ischemic CHF has not been determined obviously.[16]

The objective of the present study was to assess counts of $CD45^+CD34^+$-, $CD45^-CD34^+$-, $CD14^+CD309^+$-, and $CD14^+CD309^+Tie2^+$-phenotype circulating EPCs of various subpopulations in patients with ischemic CHF.

Methods

The study involved 153 patients (86 male) aged 48–62 years, with angiographically proven coronary artery disease (CAD) with stenotic lesion of ≥1 coronary artery >50%, and 25 healthy volunteers. CHF was diagnosed in 109 patients (71.2%) with angiographically proven CAD by means of conventional criteria according to current clinical guidelines.[17] Table 1 presents characteristics of the patients who participated in the study. All of the patients gave their written informed consents for participation in the study. The following were exclusion criteria: Q-wave and non–Q-wave myocardial infarction within 3 months before study enrollment; severe kidney and liver diseases that may affect clinical outcomes; malignancy; plasma creatinine level >440 μmol/L; estimated glomerular filtration rate (GFR) <35 mL min^{-1} 1.73 m^{-2}; brain injury within 3 months before study enrollment; body mass index (BMI) >30 kg/m^2 or <15 kg/m^2; pulmonary edema; tachyarrhythmia; valvular heart disease; thyrotoxicosis; ischemic stroke; intracranial hemorrhage; acute infections; surgery; trauma; any ischemic event in 3 previous months; inflammations within 1 previous month; neoplasm; pregnancy; implanted pacemaker; any disorder that, according to investigators, might discontinue patient's participation in the study; and patient's refusal to participate in the study or to give consent for it.

Methods for Visualization of Coronary Arteries

Multispiral computerized tomographic angiography and/or angiographic study were carried out to verify the ischemic nature of the disease in patients. Multispiral computerized tomographic angiography was carried out for all of the asymptomatic patients at high risk of CAD before study enrollment. When atherosclerotic lesions of the coronary arteries were verified, patients were subjected to conventional angiographic examination if indications for revascularization were available. CAD was considered to be diagnosed on availability of previous angiographic examinations carried out not later than 6 months before if no new cardiovascular events occurred for this period and the procedures were available for assessment.

Multispiral Computerized Tomographic Angiography

The coronary artery wall structure, as well as atheroma geometries and compositions, were measured by means of contrast spiral computerized tomographic angiography[18] on a Somatom Volum Zoom scanner (Siemens, Erlangen, Germany) with 2 detector rows when holding breath at the end of breathing in. After preliminary native scanning, Omnipak nonionic contrast (Amersham Health, Ireland) was administered for the optimal image of the coronary arteries. To reconstruct the image, 0.6-mm-width axial tomographic slices were used. Coronary artery calcification was assessed by calculating Agatston score index and by measuring the calcification mass.[19] The authors determined the presence of calcified atheromas, high-density noncalcified atherosclerotic plaques (HD-NCPs), and low density noncalcified atherosclerotic plaques (LD-NCPs). The presence of calcified atheromas was supposed at the computerized tomography density ≥+150 Hounsfield units (HU); the presence of HD-NCP was supposed at the density level within the range of +30 to +149 HU; and the presence of LD-NCP was supposed at the density level within the range of −100 to +30 HU.[19,20]

Assessment of Intracardiac Hemodynamics

Intracardiac hemodynamics was assessed by means of transthoracic ultrasonic cardiography according to a conventional procedure on an Acuson apparatus (Siemens) in B ultrasonography regimen and tissue Doppler echocardiography regimen from parasternal, subcostal, and apical positions over the short and long axis with P sensor of 5 MHz. Left ventricular end-diastolic and end-systolic volumes were measured by modified Simpson planimetric method; they were measured by cylinder method if severe failure of local myocardial contractility was verified. The left ventricular ejection fraction (LVEF) was assessed in compliance with the requirements of American Society of Echocardiography.[21] Tissue Doppler echocardiography was carried out in 4-, 3-, and 2-chamber projections in each of 16 segments of the left ventricle and in 4 spots of the mitral annulus: at the base of posterior septal, lateral, and inferior and anterior left ventricular walls.[22] Peak systolic (Sm), early diastolic (Em), and late diastolic (Am) myocardial velocities were measured in the mitral annulus area, followed by calculating the ratios of velocity of early diastolic left ventricular filling (E) to Am (E/Am) and to Em (E/Em).

Calculation of Glomerular Filtration Rate

Calculation of GFR was carried out with the use of the Modification of Diet in Renal Disease 6 formula.[23]

Measurement of Highly Sensitive C-Reactive Protein, N-Terminal Pro–B-Type Natriuretic Peptide, and Total Cholesterol and Its Fractions

To determine highly sensitive C-reactive protein (hs-CRP), N-terminal pro–B-type natriuretic peptide (NT-proBNP), total