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Circulating angiogenic cell populations, vascular function, and arterial stiffness

Susan Cheng[a,b], Na Wang[d], Martin G. Larson[a,e], Joseph N. Palmisano[d], Gary F. Mitchell[c], Emelia J. Benjamin[a,d,g,i], Ramachandran S. Vasan[a,g,i], Daniel Levy[a,f], Elizabeth L. McCabe[j], Joseph A. Vita[g,h,i], Thomas J. Wang[a,j], Stanley Y. Shaw[j,k], Kenneth S. Cohen[l,m], Naomi M. Hamburg[a,g,h,i,*]

[a] *Framingham Heart Study, Framingham, MA, United States*
[b] *Division of Cardiovascular Medicine, Brigham and Women's Hospital, Boston, MA, United States*
[c] *Cardiovascular Engineering Inc., Norwood, MA, United States*
[d] *Epidemiology Department and Data Coordinating Center, Boston University School of Public Health, Boston, MA, United States*
[e] *Department of Mathematics and Statistics, Boston University, Boston, MA, United States*
[f] *Center for Population Studies, National Heart, Lung, and Blood Institute, Bethesda, MD, United States*
[g] *Evans Department of Medicine, Boston University School of Medicine, Boston, MA, United States*
[h] *Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, MA, United States*
[i] *Preventive Medicine and Cardiology Sections, Boston University School of Medicine, Boston, MA, United States*
[j] *Cardiology Division, Massachusetts General Hospital, Boston, MA, United States*
[k] *Center for Systems Biology, Massachusetts General Hospital, Boston, MA, United States*
[l] *Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, United States*
[m] *Section of Hematology/Oncology, University of Chicago Medical Center, Chicago, IL, United States*

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ABSTRACT

Objective: Several bone marrow-derived cell populations have been identified that may possess angiogenic activity and contribute to vascular homeostasis in experimental studies. We examined the extent to which lower quantities of these circulating angiogenic cell phenotypes may be related to impaired vascular function and greater arterial stiffness.
Methods: We studied 1948 Framingham Heart Study participants (mean age, 66 ± 9 years; 54% women) who were phenotyped for circulating angiogenic cells: CD34+, CD34+/KDR+, and early outgrowth colony forming units (CFU). Participants underwent non-invasive assessments of vascular function including peripheral arterial tone (PAT), arterial tonometry, and brachial reactivity testing.
Results: In unadjusted analyses, higher CD34+ and CD34+/KDR+ concentrations were modestly associated with lower PAT ratio ($\beta = -0.052 \pm 0.011$, $P < 0.001$ and $\beta = -0.030 \pm 0.011$, $P = 0.008$, respectively) and with higher carotid-brachial pulse wave velocity ($\beta = 0.144 \pm 0.043$, $P = 0.001$ and $\beta = 0.112 \pm 0.043$, $P = 0.009$), but not with flow-mediated dilation; higher CD34+ was also associated with lower carotid-femoral pulse wave velocity ($\beta = -0.229 \pm 0.094$, $P = 0.015$). However, only the association of lower CD34+ concentration with higher PAT ratio persisted in multivariable analyses that adjusted for standard cardiovascular risk factors. In all analyses, CFU was not associated with measures of vascular function or arterial stiffness.
Conclusions: In our large, community-based sample of men and women, circulating angiogenic cell phenotypes largely were not associated with measures of vascular function or arterial stiffness in analyses adjusting for traditional risk factors.

1. Introduction

Accumulating evidence suggests that select circulating cell populations have endothelial reparative and angiogenic properties [1]. Derived from the bone marrow, these cell phenotypes have been identified using cell surface markers and culture-based assays [1]. In models of arterial ischemia and myocardial infarction, CD34+ cells are capable of promoting both neovascularization and angiogenesis [2,3]. Similarly, selectively cultured peripheral

* Corresponding author at: Whitaker Cardiovascular Institute, Department of Medicine, Boston University School of Medicine, 88 East Newton Street C818, Boston, MA 02118, United States. Tel.: +1 617 638 7260; fax: +1 617 638 8756.
E-mail address: naomi.hamburg@bmc.org (N.M. Hamburg).

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blood mononuclear cells appear to facilitate re-endothelialization and minimize neointimal formation in arterial injury models [4–6]. In humans, lesser quantity of circulating angiogenic cells has been associated with both greater burden of cardiovascular risk factors [7–9] and a higher incidence of cardiovascular events [9–12]. Thus, these cell-based phenotypes have been hypothesized to represent angiogenic potential, depletion of which predisposes to impaired vascular function and the development of vascular disease.

To elucidate the role of circulating angiogenic cell phenotypes in the development of vascular disease, several clinical studies have examined the association of these cell phenotypes with measures of vascular function. However, these prior studies have been limited to referral samples of fewer than 100 subjects each and have reported conflicting results [8,13–17]. Whereas some studies have observed a relation of reduced quantities of angiogenic cells with impaired endothelial function [8,13,14] and higher arterial stiffness [15], other studies have observed no association [16]. Therefore, we used several methods to assess circulating angiogenic cells and investigated their association with a comprehensive set of non-invasive measures of vascular function and arterial stiffness in a large, community-based sample of men and women.

2. Methods

2.1. Study sample

In 1971, the Framingham Offspring Study enrolled 5124 individuals who were either offspring of the Original cohort or spouses of offspring [18]. Offspring study participants receive routine examinations approximately every 4 years, and a total of 3021 participants attended the eighth examination cycle (2005–2008). Of this sample, 1948 had phenotyping of circulating angiogenic cells and a complete assessment of cardiovascular risk factors, in addition to available vascular function measures (brachial reactivity at the seventh examination [1998–2001], and arterial tonometry and peripheral arterial tonometry [PAT] at the eighth examination). Systolic and diastolic blood pressures were the average of two physician-measured readings. Body mass index was calculated as weight divided by height squared (kg/m^2). Blood was drawn for glucose, total and high-density lipoprotein (HDL) cholesterol, and triglycerides after overnight fast. Use of medications and cigarette smoking (regular smoker within the past year) were self-reported. Diabetes was defined as having a fasting glucose $\geq$126 mg/dL or taking medications to treat diabetes. Prevalent cardiovascular disease (CVD) was adjudicated by a panel of 3 investigators and defined as a prior history of myocardial infarction, heart failure, or stroke.

2.2. Assessment of cell phenotypes

Blood specimens were collected from fasting participants in the morning between 8 and 9 A.M. to assay the following angiogenic cell phenotypes: CD34+ cells, CD34+/KDR+ cells, and colony forming unit (CFU). Each blood specimen was initially centrifuged and the resulting buffy coat was further processed for cell phenotyping within 4 h of blood collection as previously described [8,19], with modifications. Specifically, buffy coats were diluted to 10.5 mL using PBS (Invitrogen) and then layered over 5 mL of Ficoll (Amersham Pharmacia Biotech). Each specimen was then centrifuged at 2200 rpm for 15 min at 10 °C. Using Ficoll density-gradient centrifugation, peripheral blood mononuclear cells were isolated from the buffy coat and then processed for flow cytometry and CFU assay [8].

2.2.1. Flow cytometry

Peripheral blood mononuclear cells were incubated on ice for 15 min with FcR blocking agent (Miltenyi Biotec), and then for an additional 25 min with anti-KDR PE (R&D Systems) and anti-CD34 FITC (BD Biosciences) anti-human antibodies. Specimens were washed and then fixed in 2% paraformaldehyde. Surface marker expression was evaluated by fluorescence-activated cell sorter (FACS) analysis, and positive cells were quantified using a Becton-Dickinson FacsCalibur flow cytometer using fluorochrome-matched IgG isotype controls. The frequency of CD34+ cells was identified within the nucleated cell gate using population gating; KDR+ events within the CD34 population were also analyzed via population gating. FlowJo analysis software (Treestar, Inc.) was used to quantify CD34+ and CD34+/KDR+ cells [19], where quantities of each cell type were reported as percent of the total number of gated nucleated cells. To ensure consistency, all flow analysis plots were reviewed by an investigator blinded to identity and risk factor status of the participant (KSC).

2.2.2. Colony forming unit assay

Peripheral blood mononuclear cells were washed with PBS, and remaining red blood cells were then lysed with ACK lysis buffer (Fisher Scientific). In each well of a 6 well fibronectin coated tissue culture plate (BD Biosciences), viable mononuclear cells (5 million per specimen) were plated in M199/20% FBS and cultured at 37 °C/5% CO_2. After 2 days, non-adherent cells were collected and 2 million viable cells in M199/20% FBS were re-plated in wells of a 24-well fibronectin coated tissue culture plate. After an additional 5 days of culture, the number of colonies in each well was counted by a single, blinded technician. Colony number was reported as the average number of colonies per well across up to 12 wells; in wells where the number of colonies was too numerous to count (mean of 4.7 wells from 63 individuals), the number of colonies per well was censored at 300. Following initial cell plating and colony counting, one of two technicians performed replating of all cells; to minimize the effects of operator variation, colony counts were standardized by identity of the replating technician.

2.3. Assessment of vascular function

2.3.1. Flow-mediated dilation

Fasting participants who attended the seventh examination cycle (1998–2001) underwent assessment of brachial artery flow-mediated dilation (FMD) as previously described and reported as the percent change in brachial artery diameter from its baseline value to that at 60 s after cuff deflation [20]. A subset of participants had flow assessment with Doppler to assess the hyperemic flow velocity after cuff deflation, a measure of microvessel dilation (the protocol was implemented part of the way through the examination, accounting for the smaller sample size) [20,21].

2.3.2. Peripheral arterial tonometry

PAT was assessed in fasting participants attending their eighth examination cycle (2005–2008) by measuring digital pulse amplitude using a PAT device placed on the tip of each index finger, as previously described [22]. The index finger pulse amplitude before and after hyperemia was measured in standard fashion. The ratio of post- to pre-ischemia pulse amplitudes was calculated for the hyperemic and control (contralateral, non-hyperemic) index fingers. The PAT ratio was defined as the natural log-transformed ratio of the hyperemic to the control finger in the 90–120 s period after cuff deflation [23].

2.3.3. Arterial stiffness

Non-invasive hemodynamics and measures of arterial stiffness were assessed by using arterial tonometry in fasting participants