Circulating Progenitor Cells is Linked to Cognitive Decline in Healthy Adults

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ABSTRACT

Objective: Cognitive and cardiovascular disorders share many risk factors. Higher bone-marrow derived progenitor cells (PC) in blood are associated with lower rates of cardiovascular events but the association of PC with cognitive function is unclear. The objective of this study was to assess the association between PC and cognition in a sample of healthy adults enrolled in a cohort study.

Materials and Methods: A random sample of employees at Emory University and Georgia Institute of Technology were followed for 4 years and underwent yearly vascular and cognitive assessment ($N = 430$, mean age = 49.2 years, 70% women, and 27% African-American). Cognition was assessed using computerized versions of 15 cognitive tests and principal component analysis was used for deriving cognitive scores: executive function, memory and working memory. PC were defined as mononuclear cells with specific surface markers (7 phenotypes). Decreased cognition in a domain was defined as performing below the lowest quartile for the corresponding domain at baseline. Generalized estimating equations were used to investigate associations between PC and cognition.

Results: Higher PC levels at baseline were associated with lower risk of cognitive decline in the executive and working memory domains during the follow-up period ($P < 0.002$ for all PC phenotypes). Further, the degree of decline in PC over the follow-up period was correlated with a corresponding decline in performances in all 3 cognitive domains over the same period (All $P < 0.002$).

Conclusion: Lower PC and greater yearly declines in PC are associated with greater cognitive decline. These findings suggest the role for PC in neurocognitive aging.

Key Indexing Terms: Progenitor cells; Cognitive function; Memory; Pre-eclampsia. **[Am J Med Sci 2016;351(2):147–152.]**

INTRODUCTION

Many cardiovascular disease comorbidities have been identified as potential risk factors for cognitive decline, including hypertension, diabetes, hypercholesterolemia and increased vascular stiffness.[1] Over the last decade, cumulative evidence has suggested that hematopoietic progenitor cells (PC) are released from the bone marrow in response to vascular injury and participate in endothelial healing and postnatal angiogenesis.[2] These progenitor-enriched cells are mononuclear and can be identified in the peripheral circulation by their surface antigens. Although there is considerable debate about what specific antigens describe PC, it is commonly described as these cells with the CD34 antigen and coexpression of CD133, chemokine receptor (CXCR4) and vascular endothelial growth factor receptor 2 (VEGFR2), either singly or in combination.[3,4] Some but not all PC or their subtypes mature into endothelial cells and may induce angiogenic protection via a paracrine effect.[5] Independent of their fate, increased levels of these circulating PC are linked to lower cardiovascular mortality and cardiovascular events.[6]

PC are also linked to cerebrovascular disease. Low PC levels have been reported in individuals with stroke and high levels predict better outcome poststroke.[7-9] Lower levels are also linked to greater brain white matter hyperintensities, a neuroimaging marker of vascular brain injury.[10] Preliminary evidence suggest that PC levels are decreased in individuals with Alzheimer's disease (AD) compared to age-matched controls and that lower levels correlate with lower cognitive performance in those with AD.[11] The association between PC and cognitive performance in nondemented individuals has not been evaluated.

Executive function and working memory are the most susceptible cognitive domains in those with increased vascular risk factors such as hypertension and diabetes and are likely related to vascular brain injury.[12] As lower PC may be linked to increased white matter hyperintensities, a marker of vascular-related brain injury, PC may also be linked to these vascular-prone cognitive domains.

The objective of this study was to assess the association between PC levels and cognitive performance in multiple domains in a healthy cohort of subjects. The hypotheses were that higher PC counts would be linked to lower cognitive decline and that those who demonstrate PC decline over the study period would also have greater cognitive decline over the same period.

MATERIALS AND METHODS

Study Description

This study was conducted by the Predictive Health Institute, the founder of the Center for Health Discovery and Well Being at Emory and Georgia Tech University, which recruited a cohort of healthy employees of Emory University and Georgia Institute of Technology (http://predictivehealth.emory.edu). The methods of this project have been described previously.[13] The sampling of the cohort was stratified across various departments and pay levels to obtain a representative balance of employees across faculty, Fair Labor Standards Act-exempt staff, and Fair Labor Standards Act-nonexempt staff. To be eligible, a potential participant had to be employed for 2 years and be covered by a health insurance plan. An alphabetic list of employees was generated, and every 10th employee was invited to participate. Approximately 30% agreed to be contacted for screening and 10% ultimately enrolled. Exclusion criteria were a history in the past year of hospitalization except for accidents; severe psychosocial disorder; addition of new prescription medications to treat a chronic disease (except for changes in antihypertensive or antidiabetic agents); drug abuse or alcoholism; a current active malignant neoplasm; uncontrolled or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, hematologic, infectious, inflammatory, musculoskeletal, neurologic, psychiatric or respiratory disease and any acute illness in the 2 weeks before baseline studies.

Participants were first contacted by phone and then were invited for an interview. The set of measures collected on the participants included physical measures (blood pressure, heart rate, dual energy X-ray absorptiometry, body mass index [BMI] and treadmill testing), laboratory tests (metabolic, hematologic and inflammatory markers), cardiovascular function, health behaviors, medication profiles, mental health markers and cognitive function. Participants were evaluated yearly. The Emory University Institutional Review Board approved the protocols, and informed consents were obtained from all participants.

A subgroup of participants ($n = 430$) underwent blood sampling and had phenotypical blood mononuclear cellular assessment yearly for a total of 4 times. Participants who did not provide blood samples were similar in age, sex and cognitive performance (all $P > 0.05$) but were more likely to be white ($P = 0.004$) and have higher education ($P < 0.0001$) relative to those who provided blood samples. PC were identified by surface antigen profiles of circulating blood mononuclear cell (CD45 med cells) expressing $CD34^+$, $CD133^+$, chemokine receptor ($CXCR4^+$) and vascular endothelial growth factor receptor 2 ($VEGFR2^+$) markers. PC were defined using the following 7 surface antigen profiles: total CD34+ cells, dual-positive (CD34+/CD133+, CD34+/VEGF2R+ and CD34+/CXCR4+) and triple-positive (CD34+/CD133+/VEGF2R+, CD34+/CD133+/CXCR4+ and CD34+/VEGF2R+/CXCR4+) cell populations.

Flow Cytometry

Peripheral blood mononuclear cells were analyzed for the expression of surface antigens using direct flow cytometry (BD FACS Canto II Flow Cytometer; BD Biosciences, San Jose, CA) as described previously.[14,15] A total of 300 μL of venous blood (anticoagulant: ethylenediaminetetraacetic acid) was incubated with fluorochrome-labeled monoclonal mouse antihuman antibodies, namely, FITC-CD34 (BD Biosciences), PE-VEGF2R (R&D system—also known as "kinase insert domain receptor-KDR"), APC-CD133 (Miltenyi) and PE-Cy7-conjugated anti-CXCR4 (EBioscience, clone 12G5) for 15 minutes. Red blood cells were removed by lysis in 1.5 mL of ammonium chloride lysis buffer that was added to the sample and incubated for an additional 10 min. The lysis process was stopped by adding 1.5 mL of staining medium (phosphate-buffered saline with 3% heat-inactivated serum and 0.1% sodium azide). A total of 5 million events were acquired from the Cytometer with Flowjo software (Treestar, Inc) used for subsequent analysis of accumulated data. List-mode files containing at least 3,000,000 events were collected so that analysis of rare subpopulations would contain an adequate number of events. Absolute numbers of each cell subset per milliliter were determined by multiplying the counts with the number of monocytes per milliliter of blood.

Reproducibility Testing

A total of 20 list-mode files were repeatedly analyzed on 2 occasions by 2 technicians. The percentage repeatability coefficients (%), calculated as standard deviation units of differences between pairs of measurements/mean of measurements*100, were $CD34^+$ = 2.9%; $CD34^+/CD133^+$ = 4.8%; $CD34^+/CXCR4^+$ = 6.5% and $CD34^+/CD133^+/CXCR4^+$ = 7.5%. However, $CD34^+$/VEGF2R cells and $CD34^+/CD133^+$/VEGF2R cells showed poorer reproducibility at 21.6% and 35.9%, respectively.

Cognitive Assessment

Commonly employed versions of neuropsychological measures were administered via computer to 601 participants at baseline and then yearly for a total of 4 times, using software developed by Aharonson and colleagues.[16-18] Cognitive tests included memory delayed recall, memory recognition, visual-spatial learning, spatial short-term memory, pattern recall, delayed pattern recall and recognition of pattern, executive function test, mental flexibility, digit symbol substitution test, forward and backward digit span, symbol spotting and focused and sustained attention (computerized score: 0-100% correctly adjusted for skill levels).