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Lower Circulating Progenitor cells is related to greater 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

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

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corresponding decline in performances in all 3 cognitive domains over the same period (All p<0.002).

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

Keywords

Progenitor cells; cognitive function; memory

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 post-natal 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 PCs, it is commonly described as these cells with the CD34 antigen and co-expression of CD133, chemokine receptor (CXCR-4), and vascular endothelial growth factor receptor 2 (VEGFR2), either singly or in combination.[3,4] Some but not all progenitor cells or their sub-types 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 post stroke.[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 non-demented 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] Since 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 will be linked to lower cognitive decline and that those who demonstrate PC decline over the study period will also have greater cognitive decline over the same period.

SUBJECTS/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 (FLSA)-exempt staff, and FLSA-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 anti-hypertensive or anti-diabetic agents), drug abuse or alcoholism, a current active malignant neoplasm, uncontrolled or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, hematologic, infectious, inflammatory, musculoskeletal, neurological, 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, 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, gender 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. PCs were identified by surface antigen profiles of circulating blood mononuclear cell (CD45med cells) expressing $CD34^+$, $CD133^+$, chemokine receptor ($CXCR\text{-}4^+$) and vascular endothelial growth factor receptor 2 ($VEGFR2^+$) markers. PC were defined using 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+, 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] Three hundred μl of venous blood (anticoagulant: EDTA) was incubated with fluorochrome-labeled monoclonal mouse anti-human 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 lysing buffer which was added to the sample and incubated for an additional 10 minutes. The lysis process was stopped by adding 1.5ml of staining medium (PBS with 3% heat-inactivated serum and 0.1% sodium azide). Five 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 sub-populations 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—Twenty list-mode files were repeatedly analyzed on 2 occasions by 2 technicians. The percent 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%.

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% correct adjusted for skill levels)

Cognitive scores: Orthogonal scores for cognitive domains were derived using principal component analysis with Varimax (orthogonal) rotation and Kaiser normalization to perform the exploratory factor analysis and then performed a confirmatory factor analysis by exploring the correlations and model fit of the derived factor-saved scores.[19,20] The factor analysis results are shown in Table 1. Three factors provided the best solution, explaining 90% of variance in cognitive performance. Solutions with more than 3-factors added little to the explanation of the variance. The 3 factors showed a low magnitude of correlation: Factor 1 vs 2= 0.28; Factor 1 vs 3=0.04; Factor 2 vs 3=0.02 (a value less than 0.32 suggests less than 10% variance overlap and is an accepted cutoff of distinct factors[19]). Factor 1 was predominantly loaded on tests that assess mental flexibility, executive function, set-shifting and to a lesser extent attention (executive function test, DSST, Mental flexibility, spot the symbol); Factor 2 loaded on memory: episodic, visual and spatial memory (Recall and spatial STM tests); Factor 3 loaded highest on the digit backward test suggesting it may reflect working memory. The derived scoring coefficients were then used to calculate the factor-saved scores in the sample: predominantly executive (+attention), predominantly memory, and predominantly working memory domains. The distributions of the cognitive scores were extremely skewed to-the-right, as were the raw scores, suggesting a high

performance level of the participants (probably related to the high educational levels of the sample: 18.7 (standard error=0.2) years) and a ceiling effect of the tests used. Test scores were divided in all the analyses at each visit into binomial variables: low vs high performance if a participant's score was below (or above) the lowest quartile of the corresponding cognitive domain. The baseline scores were used to define the quartile cut-offs.

Data analysis—Both cognitive scores and PC counts were included as discrete variables in the longitudinal analysis, which was conducted using binomial regression with generalized estimating equations (GEE) for repeated discrete measures. GEE is appropriate for binary repeated correlated measures and it allows for the estimation of the risk in the population. The outcomes for both analyses were low vs high cognitive performance. The predictors were PC counts included as a discrete variable with the reference level being the lower tertile for each cell line. PC classification was consistent during the follow-up period compared to the baseline assignment. The cumulative relative risk of lower vs higher cognitive performances were calculated during the follow-up by the PC levels adjusted for potential confounders.[21] A separate analysis was conducted for each cognitive score and cell phenotype. Finally the association between yearly change in PC levels and yearly change in cognitive function was investigated. We first calculated a yearly rate for both measures for each participant. Then we performed multiple regression Yearly rate of change in PC vs the yearly change in cognitive scores, adjusted for covariates. All models were adjusted for education, age, race, gender, BMI, systolic blood pressure and baseline cognitive score for the respective cognitive domain. A Bonferroni correction for multiple testing was used (with an alpha=0.05/(7 PC typesx3 cognitive domains 21)=0.002 for the cut-off of statistical significance and provided all results with a 99% confidence interval). SAS (NC, Cary) was used for these analyses.

RESULTS

Of the 430 individuals, 378 had at least 2 visits when cognitive and PC assessments were completed. Participants were followed for 4 years with a total of 2,490 person-years. There were no significant differences between those with or without follow-up by age (p=0.29), gender (p=0.16), race (p=0.06), PC (p=0.35), and the derived scores in executive function (p=0.84), memory (p=0.85) and working memory (p=0.05). Those without follow-up had higher education (20 years vs 18 years in those with follow-up, p<0.0001).

Baseline characteristics of the sample are provided in Table 2. At baseline, there were no significant differences in age, gender, race, educational levels, BMI, and hypertension status by cognitive performance in the predominantly-memory and predominantly-executive derived scores. Lower performers on the predominantly-working memory derived score were more likely to be women (80% vs 66% in high performers, p=0.007) and African Americans (41% vs 23%, p=0.002) and to have lower educational level (p=0.0005) and higher BMI (p=0.03). At baseline there were no associations between PC counts and cognitive performance. At baseline, all PC levels decreased with age (p<0.05 all). There was no difference by race (all p>0.05) but $CD34^+$ (p=0.002), $CD34^+/CD133^+$ (p=0.002) and $CD34^+/CXCR4^+$ (p=0.01) were lower in women. $CD34^+$ (p=0.006), and $CD34^+/CD133^+$

(p=0.001) were inversely associated with BMI. There were no differences in PC levels by history of hypercholesterolemia or statin use. However, there was a significant difference by hypertension category where those without hypertension or hypertension without treatment had similar levels whereas those treated for hypertension had significantly higher $CD34^+$ (p=0.004), $CD34^+/CD133^+/CXCR4^+$ (p=0.004), $CD34^+/CD133^+$ (p=0.002) and $CD34^+/CXCR4^+$ (p=0.01). During the study period, 40% developed decreased performance on the predominantly executive score, 34% on the predominantly memory score, and 34% on the predominantly working memory score.

Participants with higher PC levels at baseline were less likely to develop decreased cognitive performance in both the predominantly-executive score and the predominantly-working memory scores during the follow-up period of the study. This was true for all cell PC phenotypes and was significant after adjusting for age, gender, race, BMI, educational level, systolic blood pressure and baseline cognitive performance on the corresponding cognitive score as shown in Table 3. Bonferroni multiple test adjustments also did not alter the results. There was no association between baseline PC and memory derived scores during the follow-up. When evaluating the association between yearly change of PC over the study period and the yearly change in cognitive derived scores there was a positive correlation between the two measures. This suggests that greater increase (or decrease) in PC was associated with greater (increase) or decrease in cognitive performance. This was significant for the 3 cognitive derived scores and was significant after adjusting for age, gender, race, education, blood pressure, and baseline cognitive score. These results are shown in Table 4.

DISCUSSION

This study suggests that in a sample of healthy adults, those with higher levels of PC at baseline, relative to lower levels within the same sample, have lower risk of declines in cognitive performance in the executive and working memory domains. Furthermore, longitudinal declines in the number of PC may be linked to increased risk of cognitive decline over the same period. These effects are independent of blood pressure and other factors that might affect cognition.

Scarce evidence suggests that PC may be involved in cognitive disorders such as AD and vascular dementias.[22,23] For example, AD patients show increased senescence and reduced paracrine angiogenic activity of PC, and the addition of high concentration A-β-amyloid to a PC culture reduces PC counts and endothelial nitric oxide synthase and induced apoptosis.[24] In 29 individuals with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), PC were decreased compared to 29 matched controls, and the count correlated with performance on cognitive and motor tests.[25] This study suggests that progenitors may provide a protective effect against cognitive loss even in those without evidence of cognitive disorders or dementia.

Vascular factors that contribute to cardiovascular disease risk such as hypertension and diabetes have been also identified as factors that increase future cognitive decline. [1] The mechanisms by which these risk factors induce cognitive deficits are still not clear, but endothelial injury, neurovascular inflammation and cerebral hypoperfusion have all been

proposed as potential factors.[26] Decreased PC levels are linked to increased inflammation and poor endothelium-dependent vasodilation, measured as brachial artery flow-mediated dilation.[27] In adults with stroke, transplanted PC migrate and target the ischemic injury core and promote neo-vascularization and neuronal progenitor migration .[28] Increased blood brain barrier permeability may be a mediating factor for vascular-related cognitive decline. [29] A recent report suggests that PC can be constructed? if cultured with astrocytes to differentiate into BBB like-cells.[30] PC may also reflect regenerative capacity in the vascular system.[31] Taken together, these observations support a role for PC and the overall regenerative capacity in neurocognitive function.

The strength of this study is its longitudinal nature, the detailed phenotypic characterization, and the repeated measure design for both the cognitive and PC measures. A limitation of this study is that it included a sample of highly educated persons who are healthy, thereby limiting generalization and needing replication in other groups. . In addition, their role as a potential therapeutic modality in cognitive disorders is an important implication to these findings. Progenitor cell-directed therapies may offer a new target for cognitive impairment prevention.[32,33]

Conclusion

Lower levels of PC as baseline and greater yearly declines in PC levels are both associated with greater cognitive decline. These findings indicate a potential role for progenitor cells in neurocognitive aging

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Table 1

Factor Analysis using the 15 cognitive tests and the respective loading into the three resulting factors.

	MEAN	STD	PATTERN	PATTERN	PATTERN
Cognitive Test	***N=601***		**Factor1**	**Factor2**	**Factor3**
Delayed Memory Recall	95.59	7.93	0.56	**0.78**	0.22
Memory Recognition	97.27	6.61	0.78	0.53	0.26
Mental Flexibility	96.11	6.71	**0.95**	0.18	0.06
SPOTING The Symbol	99.50	6.23	**0.87**	0.15	0.27
Digit Symbol Substitution Test	96.11	7.18	**0.90**	0.16	0.01
Digit Span Forward	99.28	6.31	**0.85**	0.15	0.33
Digit Span Backwards	95.50	10.98	0.35	0.18	**0.86**
Executive Function Test	97.82	6.64	**0.93**	0.18	0.16
Visual-Spatial Memory	88.16	14.34	0.23	**0.97**	0.08
Visual Spatial Short Term Recall	85.50	18.33	0.12	**0.99**	0.08
Pattern Recall	97.38	6.80	0.78	0.21	0.22
Pattern Recall-Delayed	85.50	18.33	0.12	**0.99**	0.08
Pattern Recognition	96.06	7.34	0.81	0.21	0.17
Focused Attention	99.07	6.30	**0.82**	0.18	0.47
Sustained Attention	96.10	6.80	**0.89**	0.21	0.14
Variance explained:			**7.91**	**4.11**	**1.40**

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 4 iterations.

Table 2

Baseline characteristics of the study sample (N=430)

	Mean	Standard error or %
Age, years	*49.2*	0.6
body mass index , kg/m2	*28.22*	0.32
education, years	*18.2*	0.2
Sex		
Men	*129*	*30%*
Women	*301*	*70%*
Race		
% white	*288*	*67%*
% African American	*116*	*27%*
Systolic blood pressure, mm Hg	*122*	0.8
Diastolic blood pressure, mm Hg	*77*	0.6
Heart Rate, bpm	*69*	0.5
% with hypertension	*159*	*37%*
% of hypertension on antihypertensive treatment	*108*	*25%*
% on statin	*64*	*15%*
% on anti-diabetic treatment	*172*	*4%*
% diabetes	*172*	*4%*
%hypercholesterolemia	*77*	*18%*
%stroke	*9*	*2%*
% heart disease	*9*	2%
Circulating progenitor cell counts cells/μL		
$CD34^+$	1.93	0.07
$CD34^+/CD133^+/VEGF2R^+$	0.09	0.01
$CD34^+/CD133^+/CXCR4^+$	0.19	0.01
$CD34^+/VEGF2R^+/CXCR4^+$	0.39	0.02
$CD34^+/CD133^+$	1.07	0.04
$CD34^+/VEGF2R^+$	0.20	0.01
$CD34^+/CXCR4^+$	0.77	0.03

Table 3

Relative risk and 99% confidence interval (CI) for having lower performance on the three derived cognitive scores by higher tertiles of progenitor cells at baseline (relative to the lower tertile)

	Relative risk* (99% CI)of having cognitive decline over the study period in the 3 domains		
PC (cells/μl)	**memory derived score**	**executive derived score**	**Working memory score**
$CD34^+$	0.51(0.13,2.02), p=0.21	0.2 (0.06,0.61), p=0.0002	0.2(0.05,0.77), p=0.002
$CD34^+/CD133^+/VEGF2R^+$	0.61(0.15,2.52), p=0.37	0.22(0.07,0.71), p=0.0009	0.17(0.04,0.66), p=0.0008
$CD34^+/CD133^+/CXCR4^+$	0.51(0.12,2.09), p=0.22	0.22(0.07,0.7), p=0.0007	0.17(0.04,0.65), p=0.0007
$CD34^+/VEGF2R^+/CXCR4^+$	0.41(0.11,1.56), p=0.09	0.23(0.07,0.82), p=0.0028	0.21(0.06,0.76), p=0.0018
$CD34^+/CD133^+$	0.43(0.11,1.73), p=0.12	0.24(0.07,0.78), p=0.002	0.19(0.05,0.7), p=0.0011
$CD34^+/VEGF2R^+$	0.48(0.12,2.01), p=0.19	0.19(0.06,0.62), p=0.0003	0.16(0.04,0.62), p=0.0005
$CD34^+/CXCR4^+$	0.54(0.14,2.05), p=0.23	0.17(0.05,0.61), p=0.0004	0.21(0.06,0.77), p=0.002

* relative risk is derived from the binomial regression analysis with Generalized Estimating

Equations for repeated measures. The reference for the relative risk is the lower tertile of PC levels. All relative risks are adjusted for age, gender, race, BMI, education, systolic blood pressure, and baseline cognitive score. We used Boneferoni correction of all p-values are as follows: 0.05 / (3domains x 7 PC types=21 tests)=0.002.

Table 4

Association between rates of longitudinal changes in cognition vs longitudinal changes in PC over the follow-up period as described by the slope *(β)* of the regression between the 2 measures

	memory derived score	executive derived score	Working memory score
	β, 99% confidence interval[*]		
$CD34^+$	0.37 (0.23,0.50)	0.42 (0.27,0.58)	0.39 (0.24,0.54)
$CD34^+/CD133^+/VEGF2R^+$	0.17 (0.03,0.32)	0.22 (0.06,0.39)	0.19 (0.04,0.35)
$CD34^+/CD133^+/CXCR4^+$	0.19 (0.05,0.33)	0.23 (0.06,0.39)	0.20 (0.04,0.35)
$CD34^+/VEGF2R^+/CXCR4^+$	0.29 (0.15,0.43)	0.36 (0.20,0.51)	0.31 (0.15,0.46)
$CD34^+/CD133^+$	0.35 (0.21,0.49)	0.40 (0.24,0.56)	0.35 (0.20,0.51)
$CD34^+/VEGF2R^+$	0.19 (0.05,0.34)	0.23 (0.07,0.39)	0.20 (0.04,0.36)
$CD34^+/CXCR4^+$	0.31 (0.17,0.45)	0.39 (0.23,0.54)	0.35 (0.19,0.50)

[*] β= standardized correlation coefficient (and 99% confidence interval) for the regression between yearly change in cognitive score vs yearly change in PC over the study period adjusted for baseline age, gender, race, blood pressure and education. A positive beta indicate that both rates are in the same direction (all $p<0.002$)