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Lower Levels of Circulating Progenitor Cells Are Associated With Low Physical Function and Performance in Elderly Men With Impaired Glucose Tolerance: A Pilot Substudy From the VA Enhanced Fitness Trial

Thomas J. Povsic,[1,2] Richard Sloane,[3,4] Jiying Zhou,[1] Carl F. Pieper,[3,5] Megan P. Pearson,[4,6] Eric D. Peterson,[1,2] Jennifer B. Green,[1] Harvey J. Cohen,[2–4] and Miriam C. Morey[2–4,6]

[1]Duke Clinical Research Institute,
[2]Department of Medicine, and
[3]Claude D. Pepper OAIC, Duke University Health Care System, Durham, North Carolina.
[4]Geriatric Research, Education, and Clinical Center, Veterans Affairs Medical Center, Durham, North Carolina.
[5]Department of Biostatistics and Bioinformatics, Duke University Health Care System, Durham, North Carolina.
[6]Center for Health Services Research and Development, Veterans Affairs Medical Center, Durham, North Carolina.

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Address correspondence to Thomas J. Povsic, MD, PhD, Box 103208, Duke University Medical Center, Durham, NC 27708.
Email: thomas.povsic@duke.edu

Background. Aging is marked by a decline in physical function. Although the biological underpinnings for this remain unclear, loss of regenerative capacity has been proposed as one cause of the loss of physical function that occurs over time. The quantity of circulating progenitor cells (CPCs) may be one reflection of regenerative capability. We sought to determine whether certain specific CPC subpopulations were associated with physical function.

Methods. Baseline CPCs were measured in 129 randomized participants in the Enhanced Fitness clinical trial based on the cell surface markers CD34, CD133, CD146, and CD14 and aldehyde dehydrogenase (ALDH) activity. Physical function was assessed using usual and rapid gait speed, 6-minute walk distance, chair stand time, and balance time.

Results. Low counts of early angiogenic CPCs identified as $CD34^+$, $CD34^+CD133^+$, and ALDH-bright ($ALDH^{br}$) cells were associated with low usual gait speed ($p < .005$, $p < .001$, and $p < .007$), rapid gait speed ($p < .001$, $p < .003$, and $p < .001$), and 6-minute walking distance (all comparisons $p < .001$), and longer time required to complete five chair stands ($p < .006$, $p < .002$, and $p < .004$). CPC counts of mature endothelial or monocytic markers were not associated with physical function.

Conclusions. The numbers of $CD34^+$ and $ALDH^{br}$ CPCs are significantly lower in patients with impaired physical function. Further studies are needed to determine the underlying causes for this association.

Key Words: Endothelial progenitor cells—Physical performance—Physical function—Aging—Progenitor cells biology.

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AGE represents the single most critical risk factor for chronic disease and mortality. For instance, the likelihood of a cardiovascular event in a 30-year-old is 20-fold less than in a 70-year-old with similar risk factors, and the average life expectancy of a 65-year-old today is nearly 20 years, whereas a 90-year-old can expect only an additional 4 years of life (1). The associations between age, chronic disease, and physical function have been extensively studied, but the biological underpinning to these associations remains elusive.

One theory suggests that organisms possess a finite capacity for stem cell–mediated repair after chronic exposure to tissue injury. Once the reparative capacity of the stem cells is exhausted, overt clinical manifestation of disease and loss of function ensues (2). This biological theory can be integrated into a conceptual framework used in gerontology that describes a pathway from pathology to impairment, functional limitation, and disability (3). Active pathology involves a disruption of normal cellular processes and the effort of the organism to regain a normal state and can

result from multiple etiologies: infection, disease, metabolic imbalance, or other causes. Within this framework, chronic and repetitive exposure to cellular injury (active pathology) results in a cycle of stem cell mobilization and repair and eventually to impairment or loss at the tissue, organ, or system level. Functional limitations refer to the performance at the whole body level and represent restrictions in the ability of a person to perform tasks such as walking, rising from a chair, or other physical activities, which is the focus of this article. We suggest that stem cell exhaustion leads to a diminished stem cell reparative capacity, which contributes to system level impairment or impairments across multiple systems reflected as impaired physical function.

We (4,5) and others (6) have described both an age-associated depletion of circulating progenitor cells (CPCs) and an age-associated impaired mobilization response after injury (7), although these findings have not been universally replicated (8,9). Considerable evidence points to aging and chronic injury, resulting in a loss of progenitor cell responsiveness and proliferative capacity (10,11). Lower quantities of CPCs are in turn associated with poorer outcomes after acute coronary events (9). Whether similar associations are found between CPCs and physical function is not known.

CPCs have been measured on the basis of markers such as CD34 and CD133, which identify progenitors with hematopoietic and endothelial properties; however, studies have indicated that certain angiogenic progenitors may express monocytic markers (12,13) and that more mature endothelial markers may also identify CPCs capable of vascular repair (14). We have identified aldehyde dehydrogenase (ALDH) activity, a marker common to multiple progenitor cell types (15,16) as a novel marker of a circulating stem cell tied to extent of vascular disease (4,17). We measured circulating progenitors based on each marker to determine if any was associated with physical function.

The Enhancing Fitness in Older Overweight Veterans with Impaired Glucose Tolerance (Enhanced Fitness) trial (18,19) was a randomized controlled trial testing the effects of a 1-year home-based physical activity telephone counseling intervention compared with usual care on glycemic control. As part of the Enhanced Fitness trial, patients underwent rigorous assessment of physical function (20) and blood sampling, thus offering a unique opportunity to measure a variety of conventional and novel CPCs and associate these with markers of physical function. In this cross-sectional analysis, we describe the relationship of baseline CPC levels and both self-reported and objective assessments of physical function.

Methods

Enhanced Fitness Study

The Enhanced Fitness trial randomized 302 eligible patients (18,19) with impaired glucose tolerance (fasting glucose 100–125 mg/dL) and who were free from diabetes, had a hemoglobin A1c below 7%, were not on diabetes medications, and had a body mass index (BMI) between 25 and 45 kg/m^2. Individuals who exceeded current physical activity recommendations were excluded (21). Outcomes were assessed by individuals blinded to intervention status.

The research protocol was reviewed and approved by the Durham Veteran Affairs and the Duke University Medical Center institutional review board annually. Institutional review board approval for this substudy was obtained after 164 patients had enrolled in the parent study.

Baseline Variables

Demographic and biometric characteristics collected at baseline as part of the Enhanced Fitness study included age, sex, race/ethnicity, income, education level, height, weight, blood pressure, and waist circumference. Comorbidity was assessed using the Older Americans Resources & Services (OARS) Comorbidity and Symptom Index by trained researchers following rigorous validated survey methods (22). The index asks an individual to provide an affirmative or negative response to the presence of 35 unique conditions or symptoms at the present time. Self-report of medical history is considered a valid ascertainment in comparison with physician-diagnosed diseases (23).

Physical Function Measures

Physical function was assessed by directly measured tests of physical performance and self-report of health-related physical function. Usual and rapid gait speeds were measured over 2.4 m with a wireless timing device. The better of two trials for usual and rapid ("as fast as you can") tests was recorded. The 6-minute walk test was performed in a secluded corridor with participants instructed to cover as much distance as they could in 6 minutes. Tests of standing balance (ability to stand for 10 seconds with feet together, semi-tandem, and tandem) and time to complete five chair stands were performed as described by Guralnik and colleagues (24). Grip strength was measured as the best of three trials for the preferred hand using a handheld dynamometer. Self-report of function was obtained as part of a separate computer-assisted telephone interview using the physical function subscale of the Short-Form Medical Outcomes study (SF-36) questionnaire (18). Trained individuals blinded to study assignment performed each test following standardized and validated methods.

CPC Analysis

Prior to assessments of physical function, blood was collected into citrated tubes and subjected to centrifugation (1,800g × 20 min). After plasma removal, the buffy coat was transferred to a 50-mL conical tube. Cells were washed, suspended, and recollected after brief (1,800g × 5 min) centrifugation. All analyses were performed within 3 hours of sample collection.

CPC number was assayed using flow cytometry based on cell surface expression of CD133, CD34, CD14, and CD146. In a separate experiment, CPCs were identified on the basis of ALDH activity, a property inherent to endothelial and other progenitor cells (4,16).

ALDH^{br} Cell Enumeration

Total human peripheral blood was analyzed for the relative content of cells with low orthogonal light scatter and high ALDH activity content (ALDH-bright [ALDH^{br}] cells) (4). Cells (4×10^6) were aliquoted into an Aldecount tube (Aldagen Inc., Durham, NC). Immediately after addition of the cells, 500 µL was transferred to a tube containing diethylaminobenzaldehyde, a potent inhibitor of ALDH activity (10 µM). After 30 minutes at 37°C, the cells were centrifuged, placed on ice, and flow cytometry was performed.

Analysis of Cells Based on Cell Surface Marker Expression

Isolated mononuclear cells were washed and concentrated into Iscove's modified Dulbecco's medium containing 2% fetal calf serum (10^7 cells/mL). Nonspecific antibody binding was inhibited using FcR reagent (Becton Dickinson, Franklin Lakes, NJ; 10 µL × 10 minutes), and cells incubated with CD133-phycoerythrin (Miltenyi Biotec, Auburn, CA), CD34-fluorescein isothiocyanate (Miltenyi Biotec), CD146-allophycocyanin (Becton Dickinson), and CD14-phycoerythrin-cyanin7 (Becton Dickinson). Dead and dying cells were excluded using 7-amino-actinomycin D (Invitrogen, Carlsbad, CA) added just prior to flow cytometry.

Flow Cytometry

Flow cytometry was performed by trained technicians blinded to patient identity using an LSR CANTO flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Treestar, Costa Mesa, CA). Quality control measures were performed daily using BD Comp Beads (BD Biosciences).

Analysis was performed in a blinded fashion. The numbers of CD34$^+$, CD133$^+$, CD14$^+$, and CD146$^+$ cells were identified as subpopulations of a mononuclear cell gate, determined on the basis of light front and side scatter characteristics. Reported frequencies were expressed as percentages of the mononuclear cell population. Determination of the ALDH^{br} populations was performed based on a comparison of staining in the fluorescein isothiocyanate channel and absent in a control tube.

Analytical Methods

CPCs are expressed as a percentage of the total mononuclear cell population. Spearman correlations were used to explore the associations between the CPC levels and the set of baseline demographic and comorbid condition measures. To assess the associations between CPC levels and physical function outcomes, standardized z scores ([raw data − population mean]/standard deviation) were created for log-transformed CPC concentrations to allow comparison among the candidate CPCs. The association of CPCs with the functional outcomes (usual and rapid gait speed, 6-minute walk, standing balance, chair stands, the Short Physical Performance Battery, and the SF-36 Physical Function subscale) was assessed using ordinary least squares regression in a two-step modeling strategy. The first step estimated the unadjusted association for each functional outcome (dependent variable) and each standardized CPC (independent variable). In the second step, adjusted ordinary least squares models added a set of a priori selected covariates (participant age, BMI, IL-6 level and self-reported presence of arthritis, effects of stroke, circulation problems, anemia, and high blood pressure) as independent variables. In these regression models, parameter estimates reflect the association of standardized CPC level with functional measure, so that the parameter estimate is the magnitude of change of the functional measure with a 1 *SD* increase in CPC level.

Statistical significance was declared at $p < .05$. Given the exploratory nature of the aims of this study, no adjustments were made for multiple comparisons. All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Institutional review board approval for this substudy was obtained in August 2009 after which 137 of 138 enrollees in the Enhanced Fitness study consented to participation. In eight participants, sufficient blood for analysis was either not obtained or technical issues prevented CPC analysis, leaving 129 patients in this substudy.

The clinical characteristics of the CPC cohort are shown in Table 1 and mirror those of the overall Enhanced Fitness cohort (19). Patients had a mean age of 66.6 years and were almost exclusively men, given enrollment at a VA facility (98%). Significant portions of patients reported findings of arthritis, heart disease, circulation problems, and hypertension.

Measures of physical performance were also comparable with the overall cohort of patients in the Enhanced Fitness trial (18). Participants had usual walking speeds that were within the normal range for their age but were at the 30th percentile for 6-minute walk distance, indicating poor aerobic capacity for their age and appropriate for a trial that had physical inactivity as an eligibility criteria (25,26).

CPC Analysis

CPCs were analyzed based on cell surface expression of progenitor (ALDH activity, CD133, CD34), mature endothelial (CD146), and monocytic (CD14) markers.

Table 1. Baseline Characteristics of Participants (n = 129)

	Participants	Range
Age, mean (*SD*), y	66.6 (6.23)	60–81
Age ≥75, no. (%), y	20 (15.6)	
White race, no. (%)	86 (67.2)	
Male sex, no. (%)	126 (98.4)	
Some college education or trade school, no. (%)	75 (58.6)	
Arthritis, no. (%)	65 (50.8)	
Stroke, no. (%)	2 (1.6)	
Heart disease, no. (%)	34 (26.6)	
Circulation trouble in arms or legs, no. (%)	17 (13.3)	
Anemia, no. (%)	2 (1.6)	
High blood pressure, no. (%)	89 (69.6)	
Emphysema or COPD, no. (%)	13 (10.2)	
Kidney disease, no. (%)	1 (0.8)	
Health quality of life		
General health, mean (*SD*), range 0–100	66.4 (18.4)	10–100
Number of comorbidities, mean (*SD*)	3.65 (2.39)	0–11
Number of symptoms, mean (*SD*)	1.94 (1.57)	0–6
Anthropometric and biochemical, mean (*SD*)		
BMI (kg/m^2)	31.5 (3.8)	24.9–44.1
Insulin (uIU/mL)	10.33 (5.61)	1–29
Hemoglobin A1c (%)	5.87 (0.45)	4–6.6
Cholesterol (mg/dL)	174.8 (29.8)	110–248
Triglycerides (mg/dL)	138.2 (72.1)	33–530
HDL (mg/dL)	38.0 (9.3)	21–68
LDL (mg/dL)	109.3 (27.5)	51–170
HOMA-IR	1.42 (0.8)	0.12–3.75
Physical performance*, mean (*SD*)		
Gait speed: usual pace (m/s)	1.26 (0.21)	0.74–1.72
Gait speed: rapid (m/s)	1.89 (0.36)	0.92–2.71
6-Min walk distance (yd)	530 (99.6)	315.8–797.4
Chair stands, total time in s	11.3 (3.45)	1–10
Balance time (s)	9.47 (1.70)	5–27
Grip strength (kg)	35.7 (7.8)	16–54
SPPB summary score, range 4–12	11.21 (1.04)	8–12
Self-reported function, mean (*SD*)		
Physical function, range 0–100	78.6 (18.5)	25–100

Notes: BMI = body mass index; COPD = chronic obstructive pulmonary disease; HDL = high-density lipoprotein; HOMA-IR = homeostasis model of assessment-insulin resistance; LDL = low-density lipoprotein; *SD* = standard deviation; SPPB = short physical performance battery.

*Gait speeds are speeds measured during walking at usual (usual) pace and as rapidly as possible (rapid). Six-min walk distance is distance walked in 6 min. Chair stand is the time required to complete five chair stands (better fitness associated with lower value). Balance time is the time (up to 10 s) for which a patient could stand in one of three hierarchical positions as outlined (24). Grip strength is force generated using a handheld dynameter. Better fitness is represented by higher values. The SPPB summary score is developed by grading performance on usual walking speed, time for five chair stands, and balance test according to quartiles in the Established Populations for the Epidemiologic Study of the Elderly (24), thus each patient gets a score of 1–4 for each assessment.

Mean, median, and distributions of CPCs are listed in Table 2. CD34 or CD133 expression was identified in about 0.14% of cells, whereas ALDH^{br} cells were less common. About 50% of each of CD34 or CD133 single positive cells coexpressed the other marker. Cells identified based on mature endothelial markers (CD146) or monocytic markers (CD14) were more common, and overlap with the progenitor cell markers CD34 or CD133 was less frequent. As is commonly observed, CPCs were not normally distributed with a "right-shift" in distribution toward higher CPC numbers.

Table 2. Distribution of CPC Numbers (% of mononuclear cells)

	Median (25th, 75th)	Range	Mean (*SD*)
CD34	0.143 (0.010, 0.019)	0.022–0.52	0.156 (0.083)
CD133	0.135 (0.09, 0.20)	0.019–0.574	0.155 (0.094)
CD146	0.83 (0.58, 1.16)	0.235–3.59	0.931 (0.502)
CD14	19.17 (13.3, 24.0)	0.96–46.5	19.61 (9.07)
CD133–CD34	0.056 (0.037, 0.080)	0.006–0.22	0.066 (0.41)
CD133–CD146	0.007 (0.004, 0.012)	0.001–0.053	0.011 (0.011)
CD133–CD14	0.017 (0.009, 0.041)	0.001–0.238	0.033 (0.040)
CD34–CD146	0.004 (0.002, 0.006)	0–0.055	0.005 (0.006)
CD34–CD14	0.010 (0.004, 0.018)	0–0.075	0.014 (0.013)
CD146–CD14	0.15 (0.072, 0.23)	0–1.148	0.184 (0.164)
ALDH^{br}	0.067 (0.042, 0.107)	0.007–0.399	0.083 (0.062)

Notes: ALDH^{br} = aldehyde dehydrogenase-bright; *SD* = standard deviation.

Correlation With Clinical Factors

We assessed the association of a variety of CPC numbers with clinical factors. In this exclusively elderly (minimum age 60) and overweight/obese (minimum BMI 25 kg/m^2) population, there was no correlation of CPCs with age or self-reported medical conditions including arthritis, stroke, heart trouble, circulation trouble, high blood pressure, or anemia. CPCs were also not associated with baseline levels of insulin, total cholesterol, or low-density lipoprotein cholesterol.

Levels of CD14$^+$ ($r = -.18$, $p < .05$), CD133$^+$CD146$^+$ ($r = -.24$, $p = .006$), CD133$^+$CD14$^+$ ($r = -.25$, $p = .005$), and CD34$^+$CD146$^+$ ($r = -.22$, $p = .01$) cells were lower in more obese patients, whereas CD34$^+$CD133$^+$ and ALDH^{br} cells ($r = -.18$, $p < .05$ and $r = -.17$, $p < .05$, respectively) were lower in those with self-reported anemia, although only two patients reported such a history.

Association of CPC Level With Physical Function Outcomes

Circulating immature endothelial and hematopoietic progenitors (CD34$^+$, CD34$^+$CD133$^+$, and ALDH^{br} cells) were consistently statistically associated ($p < .05$) with measures of mobility and endurance (usual gait speed, rapid gait speed, 6-minute walk distance, chair stand time, and SF-36 physical function score; Table 3). CPCs were not associated with grip strength or balance assessments. Immature progenitors defined by CD133 expression were also lower in subjects with decreased usual gait speed, 6-minute walk distance, and SF-36 physical function scores and tended to be lower in patients with impaired rapid gait speed and time for chair stands. Monocytic (CD14+) and mature endothelial (CD146+) CPCs were not associated with any of the physical function assessments (data not shown).

These associations remain when adjusting for potential confounders including age, BMI, self-reported presence of

Table 3. Association of CPC Types With Assessments of Physical Function Corrected for Age, BMI, Comorbid Conditions, and IL-6 Level

	$CD34^+$				$CD133^+$				$CD34^+CD133^+$				$ALDH^{br}$			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*		Unadjusted		Adjusted*		Unadjusted		Adjusted*	
	Estimate	*p* Value	Estimate	*p* Value	Estimate	*p* Value	Estimate	*p* Value	Estimate	*p* Value	Estimate	*p* Value	Estimate	*p* Value	Estimate	*p* Value
Usual gait speed (m/s)	0.055	.005	0.046	.015	0.040	.043	0.041	.029	0.055	.005	0.054	.005	0.052	.008	0.050	.0101
Rapid gait speed (m/s)	0.092	.007	0.079	.020	0.057	.10	0.061	.069	0.078	.022	0.074	.03	0.102	.003	0.099	.004
6-Min walk distance (ft)	90.6	.004	71.7	.012	55.6	.081	57.4	.045	75.0	.018	69.3	.017	91.9	.004	81.4	.005
Time for five chair stands (s)	−0.66	.031	−0.50	.10	−0.56	.066	−0.49	.11	−0.74	.016	−0.65	.034	−0.93	.002	−0.89	.004
Balance time (s)	0.188	.25	0.124	.40	0.042	.80	0.023	.68	0.223	.17	0.135	.37	0.138	.40	0.093	.54
Grip strength	0.663	.33	0.743	.26	0.967	.16	1.04	.11	0.931	.17	0.781	.24	0.17	.80	0.273	.69
SPPB summary score	0.211	.073	0.172	.15	0.123	.30	0.116	.33	0.245	.036	0.215	.070	0.314	.007	0.333	.005
SF-36 physical performance score	4.38	.009	3.07	.045	3.56	.036	3.30	.029	4.16	.014	3.57	.020	4.15	.014	3.36	0.031

Notes: Parameter estimates reflect the association standardized CPC level with functional measure, so that the parameter estimate is the magnitude of change of the functional measure with a 1 *SD* increase in CPC level. $ALDH^{br}$ = aldehyde dehydrogenase-bright; SF-36 = Short-Form Medical Outcomes study (SF-36) questionnaire; SPPB = short physical performance battery.

*Adjusted for age, BMI, and the set of self-reported comorbid conditions (arthritis, effects of stroke, circulation problems, anemia, high blood pressure, and IL-6 level).$ALDH^{br}$ indicates aldehyde dehydrogenase-bright; SF-36, Short-Form Medical Outcomes study (SF-36) questionnaire; SPPB, Short Physical Performance Battery.

conditions associated with impairments in physical function (including arthritis, effects of stroke, circulation problems, anemia, and high blood pressure), and IL-6, a marker of inflammation (Table 3). After correcting for confounders, ALDH^{br} cells were the most tightly associated with impaired physical function.

Discussion

Association of CPCs and Physical Function

Declining physical performance and function are normal chronological components of aging. Any number of factors—environmental, behavioral, or medical—can contribute to an acceleration of this process. We sought to evaluate whether one measure of reparative capacity, the numbers of CPCs, is associated with diminished physical function. We demonstrate that key primitive angiogenic and hematopoietic CPCs are depleted in patients with lower measures of physical capacity, particularly those measures tied to mobility and cardiorespiratory endurance.

Several elements of our observations deserve comment. First, the strongest associations between CPCs and assessments of physical function were observed for 6-minute walk distance and rapid gait speed. The lack of an association between measures of balance and CPCs suggests greater cardiorespiratory system involvement than neurologic.

Second, our study participants, although overweight and prediabetic, were largely functionally healthy community dwellers with measures of physical function that were above established thresholds for physical impairment except for aerobic capacity. These observations suggest that CPC depletion may occur prior to and perhaps contribute to clinical deterioration, while raising the possibility that measurement of CPCs may identify patients at higher risk for future loss of mobility-related physical functioning.

Third, our observations in stable patients, even when corrected for age, BMI, and self-reported presence of cardiovascular symptoms and conditions, suggest that depletion of CPCs may represent a common pathway that leads to loss of aerobic capacity and subsequent development of overt vascular disease (4,9). Further study will be needed to define the cause and effect relationship between CPC depletion, aerobic capacity, physical function, and hard endpoints including mortality, but given the highly predictive nature of physical function measures with incident hospitalization and other disabling events, falls, and death, our observations lend credence to the hypothesis that loss of stem cell reparative capacity plays a key role in aging as represented by loss of physical functioning reserve and risk for subsequent morbidity and mortality.

CPCs and Baseline Clinical Factors

We did not observe an association of any CPC types in any consistent manner with age, self-reported indices of disease, or biochemical variables. The lack of relationship with age may in part be due to the limited age range of patients enrolled as stipulated by the minimum age requirement in the Enhanced Fitness trial. The lack of an association between CPCs and self-reports of individual diseases may be due to insufficient power associated with any individual condition and CPCs. However, as noted in our theoretical framework, functional limitations are likely multicausal and due to the combined burden of one or more conditions rather than one alone.

Comparison of CPC Types

We investigated whether a combination of progenitor and endothelial markers may identify a CPC uniquely correlated with physical function, including those of monocytic (CD14^{+}) origins (12) or those of definitive endothelial lineage (CD146^{+}). Perhaps not surprisingly given that these markers identify much larger cell populations (Table 2), a preponderance of which are mature nonprogenitors, no compelling correlations with clinical factors, or measures of physical function were observed. In contrast, primitive angiogenic CPCs identified based on ALDH activity or CD34 expression were closely correlated with physical function, suggesting that future work could simplify CPC identification by emphasizing the clinical importance of circulating CD34^{+} cells or populations that contain significant CD34^{+} representation (ALDH^{br} cells).

Correlation With Other Observations

Observations from other populations have suggested that a variety of conditions such as diabetes, coronary artery disease, and incident cardiovascular events are characterized by metabolic derangements (27,28). The observations in this article parallel a prior novel mechanistic observation reported by our laboratory in which we observed an inverse association between measures of mobility and endurance, but not balance or upper extremity function, with circulating plasma acylcarnitines, particularly long and medium chained (29). The consistency of results between these studies suggests an interrelationship between metabolomics alterations and progenitor cell biology.

Limitations

The population studied consisted almost exclusively of elderly male veterans who were poorly conditioned. The applicability to other populations will need to be independently verified.

This work demonstrates an association of low CPC counts with low physical functioning. Although we proposed a theoretical framework to explain this association, the cross-sectional nature of this study limits our ability to examine the role of stem cell exhaustion as the biological underpinnings of this relationship—whether CPC loss leads to or is a result of loss of physical capability, or whether the ability

to remain physically active acts as a stimulus to further CPC mobilization can be examined in future longitudinal and interventional investigations. We corrected for baseline demographic factors that might confound the relationship of CPCs and assessment of physical function, including assessments of cardiovascular and arthritic conditions; however, it is also likely that CPCs and physical function are each related to unmeasured factors that might explain our observations. For instance, anemia and renal insufficiency have both been associated with lower CPC levels and impairments in physical function. Although the degree of self-reported anemia and renal disease was low (1.6% and 0.8%, respectively), the subclinical changes in hematopoietic or renal function remain potential unmeasured confounders and their role requires further study. We have attempted to correct for conditions such as age and self-reported cardiovascular disease; nonetheless, it is not possible nor would it be valid to correct for all such potential confounders, some of which may remain unknown. Finally, given the cross-sectional nature of this study, it is not possible to infer a temporal relationship between loss of CPCs and loss of physical function.

Our work sets the stage for future research aimed to answer such interesting and important questions. If verified in other settings, this observation would add to the growing body of evidence that progenitor cell depletion is a potential mechanistic explanation for biological aging.

Conclusions

We demonstrated that lower CPC levels are associated with lower physical function, an association that remained robust across several similar early angiogenic cell types analyzed independently and multiple measures of physical function, correcting for age and other clinical factors. These findings are novel; to our knowledge, we are the first to report this association and it raises intriguing questions about the role CPC depletion plays in age-related loss of physical functioning. Additional work defining the relationship of CPCs with physical function and change in functional reserve over time is of interest.

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