Myocardial Ischemia and Mobilization of Circulating Progenitor Cells

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Background—The response of progenitor cells (PCs) to transient myocardial ischemia in patients with coronary artery disease remains unknown. We aimed to investigate the PC response to exercise-induced myocardial ischemia (ExMI) and compare it to flow mismatch during pharmacological stress testing.

Methods and Results—A total of 356 patients with stable coronary artery disease underwent 99mTc-sestamibi myocardial perfusion imaging during exercise (69%) or pharmacological stress (31%). CD34$^+$ and CD34$^+$/chemokine (C-X-C motif) receptor 4 PCs were enumerated by flow cytometry. Change in PC count was compared between patients with and without myocardial ischemia using linear regression models. Vascular endothelial growth factor and stromal-derived factor-1α were quantified. Mean age was 63±9 years; 76% were men. The incidence of ExMI was 31% and 41% during exercise and pharmacological stress testing, respectively. Patients with ExMI had a significant decrease in CD34$^+$/chemokine (C-X-C motif) receptor 4 (-18%, $P=0.01$) after stress that was inversely correlated with the magnitude of ischemia ($r=-0.19$, $P=0.003$). In contrast, patients without ExMI had an increase in CD34$^+$/chemokine (C-X-C motif) receptor 4 (14.7%, $P=0.02$), and those undergoing pharmacological stress had no change. Plasma vascular endothelial growth factor levels increased (15%, $P<0.001$) in all patients undergoing exercise stress testing regardless of ischemia. However, the change in stromal-derived factor-1α level correlated inversely with the change in PC counts in those with ExMI ($P=0.03$), suggesting a greater decrease in PCs in those with a greater change in stromal-derived factor-1α level with exercise.

Conclusions—ExMI is associated with a significant decrease in circulating levels of CD34$^+$/chemokine (C-X-C motif) receptor 4 PCs, likely attributable, at least in part, to stromal-derived factor-1α–mediated homing of PCs to the ischemic myocardium. The physiologic consequences of this uptake of PCs and their therapeutic implications need further investigation. (***J Am Heart Assoc***. 2018;7:e007504. DOI: 10.1161/JAHA.117.007504.)

Key Words: coexpression of chemokine receptor 4 • ischemia • progenitor cell • stromal-derived factor • vascular endothelial growth factor

Repetitive episodes of transient myocardial ischemia are associated with adaptive processes that include increased collateral formation.[1,2] These processes involve recruitment of local and bone marrow–derived progenitor cells (PCs) that contribute to collateral formation and vascular regeneration.[3–7] CD34$^+$ mononuclear cells from the human bone marrow include distinct lineages of both hematopoietic (CD34$^+$/CD45^{med}) and nonhematopoietic progenitors.[8]

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Clinical Perspective

What Is New?

- Physical exercise stimulates release of bone marrow progenitor cells in patients with coronary artery disease; this is at least in part attributable to the release of vascular endothelial growth factor.
- In those with myocardial ischemia, there is a reduction in circulating progenitors, likely because of homing of the progenitors to the ischemic myocardium.
- Myocardial ischemia stimulates release of stromal-derived factor-1α, and its circulating level correlates with the decline in myocardial ischemia–dependent decrease in circulating progenitor cells.

What Are the Clinical Implications?

- Exercise stimulates release of progenitor cells from the bone marrow.
- In those with myocardial ischemia, release of stromal-derived factor-1α from the ischemic myocardium leads to homing of these cells to the ischemic myocardium in proportion to the magnitude of ischemia.
- This suggests that ischemia-mediated homing of progenitor cells promotes repair and regeneration and is a potential therapeutic target for management of patients with myocardial ischemia.

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CD34$^+$ cells have greater myocardial reparative potential than unselected populations.[9] Coexpression of chemokine (C–X–C motif) receptor 4 (CXCR4$^+$) that promotes homing of PCs to stromal-derived factor (SDF)–enriched hypoxic environments further characterizes PCs with a capacity for vascular repair.[10] In addition, cell therapy using autologous CD34$^+$ PCs for the treatment of advanced heart and peripheral vascular diseases has shown encouraging results.[11–15]

The role of circulating PCs in vivo and their ability to home to ischemic myocardium in patients with coronary artery disease (CAD) have not been well studied before.[16] Herein, we assessed the level of circulating PCs in the setting of myocardial ischemia provoked by exercise stress testing. We compared it with controls without exercise-induced ischemia and those with reversible perfusion defects during pharmacological stress testing who represent mismatch in the flow. We have also assessed the role of 2 well-known mobilizing factors of PCs, vascular endothelial growth factor (VEGF) and SDF-1α. VEGF enhances PC mobilization from their niches in the bone marrow and their proliferation.[17,18] SDF-1α, also known as CXCL12, is expressed in ischemic tissues as a result of activation of hypoxia-inducible factor-1 and plays a key role in recruitment and homing of stem cells, particularly those expressing CXCR4 that binds to SDF-1α.[19–22] Our hypothesis was that exercise stress would mobilize circulating PCs from the bone marrow into the peripheral blood and that PCs will home to the ischemic myocardium in patients with myocardial ischemia.

Methods

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Study Population

Patients were enrolled into the MIPS (Mental Stress Ischemia Prognosis Study), a prospective study that recruited patients with stable CAD, between June 2011 and August 2014, at Emory University affiliated hospitals.[23] The presence of CAD was defined by an abnormal coronary angiogram demonstrating evidence of atherosclerosis with at least luminal irregularities, documented previous percutaneous or surgical coronary revascularization, documented myocardial infarction, or a positive nuclear stress test result. Patients with acute coronary syndromes or decompensated heart failure during the previous 2 months, end-stage renal disease, or unstable psychiatric conditions were excluded. Clinical information, including previous CAD events, CAD risk factors, coronary angiography results, and current medications, was documented using standardized questionnaires and medical record reviews. The research protocol was approved by the Institutional Review Board, and all participants provided informed consent. Patients were tested in the morning after a 12-hour fast. Antianginal medications (β-blockers, calcium channel blockers, and long-acting nitrates), xanthine derivatives, and caffeine-containing products were withheld for 24 hours before stress testing.

Conventional Stress Testing

Conventional stress testing was performed using the Bruce Protocol, and when contraindicated, pharmacological testing was performed with regadenoson. The radioisotope injection was given at peak exertion during the exercise test or immediately after the regadenoson injection. Exercise was continued for at least 1 minute after the injection.

Myocardial Perfusion Imaging and Single-Photon Emission Computed Tomography Image Interpretation

Myocardial perfusion imaging with 99mTc-sestamibi single-photon emission computed tomography was performed at rest and 30 to 60 minutes on a separate day (1±3 days from the resting scan) after conventional (exercise/

pharmacological) stress, according to standard protocols. Images were interpreted by 2 experienced readers blinded to the stressor (exercise/pharmacological) and without prior knowledge of the severity of CAD or other patient medical history. Discrepancies in the interpretation of single-photon emission computed tomography images were resolved by consensus. Rest and stress images were visually compared for number and severity of perfusion defects using a 17-segment model. Each segment was scored from 0 to 4, with 0 being normal uptake, and 4 being no uptake. Ischemia was defined as a new impairment with a score of ≥2 in any segment or as worsening of a preexisting impairment by at least 2 points if in a single segment or by at least 1 point if in ≥2 contiguous segments.[24] In addition to individual segment scores, we calculated the magnitude of the ischemic myocardium as (summed difference score/68)×100, as previously described.[25]

PC Measurements

We measured circulating PC counts using flow cytometry at rest (n=564) and 45 minutes after either exercise (n=245) or pharmacological (n=111) stress testing.[26] After an overnight fast, venous blood was collected via a peripheral vein into EDTA tubes and processed within 4 hours.[26] Mononuclear cells that were CD45med and expressed CD34 and CXCR4 were enumerated. We incubated 300 μL of peripheral blood (anticoagulant, EDTA) with fluorochrome-labeled monoclonal antihuman mouse antibodies (namely, 15 μL fluorescein isothiocyanate–CD34 [BD Biosciences], 15 μL PerCP-CD45 [BD Biosciences], 3 μL PE-Cy7–conjugated anti-CXCR4 [EBioscience, clone 12G5], and 10 μL APC-CD133 [Miltenyi]) in the dark for 15 minutes. Thereafter, 1.2 mL of ammonium chloride lysing buffer was added to lyse red blood cells. Then, 1.2 mL of staining medium (PBS with 3% heat-inactivated serum and 0.1% sodium azide) was added to stop the lysing reaction. After mixing gently, samples were centrifuged at 1500 rpm for 5 minutes and then washed with PBS. Thereafter, cells were suspended in 500 μL of staining medium and mixed and run on flow cytometer within 4 hours (BD FACS Canto II Flow Cytometer). Before flow cytometry, 100 μL of AccuCheck Counting Beads (Invitrogen, catalog number PCB100) was added to act as an internal standard for direct estimation of the concentration of target cell subsets. At least 2.5 million events were acquired from the cytometer. Flow data were analyzed with Flowjo software (Treestar, Inc). Absolute mononuclear cell count was estimated as the sum of lymphocytes and monocytes using a Coulter ACT/Diff cell counter (Beckman Coulter). CD45^{med} cells are also referred to as CD45^{dim} cells, and their selection excludes CD45^{bright} and CD45$^-$ (negative) cells. By excluding these CD45$^-$ cells, we exclude nonhematopoietic progenitors.

By excluding the rare CD45^{bright} cells, we exclude lymphoblasts.

Chemokine Measurements

Venous blood samples were collected for chemokine assays at rest (n=553) and 45 minutes (n=297) after the exercise stress test. Plasma was collected into ice-cooled citrate tubes and immediately centrifuged at 4°C; obtained plasma was snap frozen at −70°C until further processing. We used the electrochemiluminescence MesoScale system (Meso Scale Diagnostics, Rockville, MD) using the SECTOR Imager 2400 to quantitate VEGF and SDF-1α levels. Lower limits of detection were as follows: VEGF, 1.12 pg/mL; and SDF-1α, 27.8 pg/mL. The midpoint calibrator interassay coefficients of variation were 3.79% for SDF-1α and 4.26% for VEGF. The intra-assay coefficients of variation were 3.40% for SDF-1α and 2.05% for VEGF.

Statistical Analysis

Student *t* tests or ANOVA test for continuous variables and χ^2 test for categorical variables were used to examine differences between assigned groups (positive versus negative ischemia). PCs are reported as median (interquartile range). Wilcoxon signed rank test was used to compare PCs and chemokine levels before and after stress testing. The base-2 logarithm transformation was used for nonnormally distributed variables (PCs and chemokines) to calculate percentage change with stress testing. Linear regression models were used to compare the change of PCs after stress test (exercise and pharmacological) in patients with and without myocardial ischemia. Simple and multiple linear regression models were used to examine the association between PCs and burden of myocardial ischemia. Spearman rank correlations were used to examine the correlation between PC change and chemokine level changes before and after stress testing. ANCOVA was used to compare the PC responses between patients with and without ischemia after adjusting for factors that correlated in bivariate analysis (*P*<0.2), with the change in PCs including age, sex, race, body mass index, previous myocardial infarction, diabetes mellitus, hypertension, and renal function.

Results

Of the 356 patients recruited (age, 63±9 years; 76% men), 69% underwent exercise stress testing and the remainder had pharmacological stress tests. Patients undergoing pharmacological stress testing were more likely to be nonwhite, with higher body mass index, more cardiovascular risk factors, lower ejection fraction, and a higher incidence of myocardial ischemia (41% versus 31%) compared with those undergoing exercise stress testing (Table 1). There were no significant

differences between patients with and without myocardial ischemia during either type of stress test, except for SDF-1α (Table 1).

Changes in Circulating PC Counts With Stress Testing

There were no significant differences in the resting PC counts between patients who had exercise versus pharmacological stress testing, nor between patients with and without ischemia during either stress test (Table 1). Overall, there was no significant change in PC counts 45 minutes after either stress test; however, there was a significant increase in the hemoglobin level after the exercise stress test (Table 2).

Changes in Circulating PC Counts During Stress Testing in Patients With and Without Myocardial Ischemia

Among patients who had exercise stress testing, there was a significant difference in the change in circulating numbers of CD34+ cells ($P=0.007$) and CD34+/CXCR4+ cells ($P=0.001$) with exercise among those who developed myocardial ischemia compared with those with a negative stress test result (Figure 1, Table 2). Thus, although the CD34+ cell count remained unchanged (-5.5%, $P=0.3$) and the CD34+/CXCR4+ decreased by 18% ($P=0.01$) in patients who developed exercise-induced ischemia, they increased by 9% ($P=0.007$) and 14.7% ($P=0.02$), respectively, in patients without exercise-induced ischemia. The changes in CD34+ and CD34+/CXCR4+ PC counts in patients with and without exercise-induced myocardial ischemia remained significant, even after adjustment for age, sex, race, body mass index, history of myocardial infarction, hypertension, diabetes mellitus, and renal function.

There was a significant negative correlation between the magnitude of the single-photon emission computed tomography ischemic defect during exercise stress testing and the decrease in circulating CD34+/CXCR4+ cells (Pearson $ρ=-0.19$, $P=0.003$). Thus, for each 1% increase in the ischemic defect size, there was a 2.2% decrease in circulating CD34+/CXCR4+ PC levels.

The observed change in the total CD34+ cell count was driven by the change in the CD34+/CXCR4+ cells. No significant change was observed in CD34+/CXCR4negative cells in patients with or without ischemia. Moreover, when both CD34+ and CD34+/CXCR4+ cells were analyzed in the same model, only the change in CD34+/CXCR4+ cells was independently associated with exercise-induced ischemia. There were no significant differences in the change in CD34+/CD133+ cells among those with compared with those without myocardial ischemia (Table 3).

Moreover, and in contrast with the observations during exercise, there were no significant changes in PC counts between patients with and without myocardial flow mismatch detected during the pharmacological stress testing (Figure 1, Tables 2 and 3).

Chemokine Changes During Exercise Stress Testing

Resting levels of VEGF and SDF-1α were not significantly different in patients with or without myocardial ischemia during either stress test (Table 1).

Plasma VEGF levels increased similarly (15%, $P<0.001$) in all patients undergoing exercise stress testing, regardless of the presence or absence of ischemia (Table 2). The change in SDF-1α was significantly different among those with and without exercise-induced ischemia ($P=0.01$); SDF-1α level decreased (-6.2%, $P<0.01$) in those without ischemia, but it remained unchanged in those developing ischemia (1%, $P=0.38$). More important, the change in SDF-1α level correlated inversely with the change in CD34+ and CD34+/CXCR4+ PCs in those with exercise-induced ischemia ($P=0.03$) (Table 4, Figure 2), suggesting that patients with the greatest increase in SDF-1α after exercise had the greatest reduction in circulating PCs. Figure 2 demonstrates the relationship between the change in circulating SDF-1α level and the change in reduction in circulating PCs.

Discussion

In the largest study to date that assesses the effect of cardiovascular stress testing on PCs in peripheral blood, we demonstrate that there is a significant decrease in circulating CD34+/CXCR4+ PC counts after the development of exercise-induced myocardial ischemia. Furthermore, a greater burden of ischemic myocardium was associated with a proportionately greater decrease in circulating CD34+/CXCR4+ PCs. In contrast, in patients without exercise-induced ischemia, circulating PC counts increased, and no changes were observed in patients undergoing pharmacological stress testing. The change in circulating SDF-1α level was significantly greater among patients with compared with those without myocardial ischemia during exercise. Moreover, the decrease in PC counts in those with exercise-induced ischemia correlated inversely with the change in SDF-1α levels. Because PCs expressing the CXCR4 epitope have the ability to home to areas of ischemia with increased expression of its ligand, SDF-1α, our results suggest that the increased expression of SDF-1α from the ischemic myocardium promotes homing of the CD34+/CXCR4+ PCs, resulting in a decrease in circulating levels of PCs. Previous studies have demonstrated that homing of CD34+/CXCR4+ PCs

Table 1. Demographic and Clinical Phenotypes in Patients With and Without Ischemia During Exercise and Pharmacological Stress Testing

Variable	Exercise Stress				Pharmacological Stress				
	All Subjects (n=245)	Negative for Ischemia (n=168)	Positive for Ischemia (n=77)	P Value*	All Subjects (n=111)	Negative for Ischemia (n=66)	Positive for Ischemia (n=45)	P Value*	P Value†
Age, y	62.2±8.6	61.9±8.9	62.9±8	0.47	62.8±9.7	63.5±9.8	61.9±9.6	0.38	0.58
Male sex	191 (77.96)	127 (75.6)	64 (83.12)	0.19	76 (68.47)	44 (66.67)	32 (71.11)	0.62	0.1
White race	181 (73.88)	127 (75.6)	54 (70.13)	0.37	65 (58.56)	41 (62.12)	24 (53.33)	0.36	0.001
Body mass index, kg/m²	29.6 (5.2)	29.5 (5.4)	29.7 (4.6)	0.53	30.7 (5.2)	30.6 (5.1)	30.9 (5.5)	0.83	0.03
Hypertension	178 (72.65)	119 (70.83)	59 (76.62)	0.35	92 (82.88)	53 (80.3)	39 (86.67)	0.38	0.03
Dyslipidemia	200 (81.63)	134 (79.76)	66 (85.71)	0.26	91 (81.98)	52 (78.79)	39 (86.67)	0.29	0.92
Diabetes mellitus	68 (27.76)	47 (27.98)	21 (27.27)	0.91	44 (39.64)	25 (37.88)	19 (42.22)	0.65	0.01
Smoking	139 (56.97)	93 (55.69)	46 (59.74)	0.55	77 (69.37)	47 (71.21)	30 (66.67)	0.61	0.04
Myocardial infarction	91 (37.14)	65 (38.69)	26 (33.77)	0.46	34 (30.63)	21 (31.82)	13 (28.89)	0.74	0.23
Creatinine clearance, mL/min per 1.73 m²	82.7±20.8	81.8±20.2	84.8±22	0.34	81±22.3	79.4±22.7	83.2±21.7	0.19	0.56
Ejection fraction	53.9±11.3	54.3±11	52.9±12	0.33	46.5±15.7	46.9±16	45.9±15.5	0.95	<0.001
Medications									
Aspirin	222 (90.61)	151 (89.88)	71 (92.21)	0.56	83 (75.45)	51 (77.27)	32 (72.73)	0.59	<0.001
ACE inhibitors	121 (49.39)	77 (45.83)	44 (57.14)	0.10	49 (44.55)	27 (40.91)	22 (50)	0.35	0.36
ARBs	38 (15.51)	28 (16.67)	10 (12.99)	0.46	24 (21.82)	13 (19.7)	11 (25)	0.51	0.16
β Blocker	172 (70.2)	118 (70.24)	54 (70.13)	0.99	92 (83.64)	55 (83.33)	37 (84.09)	0.92	0.007
Clopidogrel	89 (36.33)	58 (34.52)	31 (40.26)	0.39	42 (38.18)	21 (31.82)	21 (47.73)	0.09	0.76
Statins	215 (87.76)	145 (86.31)	70 (90.91)	0.31	90 (81.82)	55 (83.33)	35 (79.55)	0.61	0.15
Leukocyte counts, ×10³ cells/μL									
White blood cells	5.9±1.7	6±1.8	5.7±1.5	0.22	6.4±2	6.2±1.7	6.6±2.4	0.47	0.05
Absolute mononuclear cell count	0.4±0.3	0.4±0.3	0.4±0.2	0.61	0.5±0.4	0.5±0.3	0.5±0.5	0.81	0.16
Hemoglobin, g/dL	14±1.5	13.9±1.4	14.2±1.5	0.08	13.7±1.7	13.7±1.5	13.7±1.9	0.76	0.07
Resting progenitor cells, median (IQR), cells/mL									
CD34+	1596 (1049–2423)	1609 (1110–2430)	1467 (1006–2423)	0.81	1646 (1027–2142)	1662 (1120–2078)	1582 (1013–2247)	0.91	0.86
CD34+/CXCR4+	674 (360–1031)	689 (366–1043)	673 (333–970)	0.52	625 (412–1102)	644 (431–1139)	623 (412–964)	0.78	0.63
CD34+/CD133+	745 (462–1147)	757 (499–1161)	690 (430–1139)	0.40	748 (445–1064)	740 (489–1013)	765 (418–1183)	0.90	0.69
Resting chemokines, median (IQR), pg/mL									
VEGF	52 (37–76)	52 (36–76)	53 (38–76)	0.61	51 (38–83)	56 (42–88)	46 (36–65)	0.57	0.76
SDF-1α	1162 (968–1430)	1162 (971–1430)	1169 (946–1449)	0.49	1265 (1010–1553)	1280 (1040–1555)	1261 (1006–1472)	0.03	0.18

Data are presented as number (percentage) or mean±SD unless otherwise indicated. Progenitor cell count was presented as median (IQR), giving a skewed distribution. ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; IQR, interquartile range; SDF-1α, stromal-derived factor-1α; and VEGF, vascular endothelial growth factor.
*P value comparing between patients with and without ischemia.
†P value comparing patients undergoing exercise vs pharmacological stress test.

Table 2. Changes in Progenitor Cells, Chemokines, and Peripheral Blood Cell Counts During Stress Testing

Variable	Exercise Stress (n=245)				Pharmacological Stress (n=111)			
	All Subjects	Negative for Ischemia	Positive for Ischemia	P Value*	All Subjects	Negative for Ischemia	Positive for Ischemia	P Value*
Progenitor cells, cells/mL								
CD34⁺ cells	6 (−377 to 362)	54 (−295 to 509)	−155 (−529 to 275)†	0.007	7 (−418 to 476)	49 (−380 to 595)	−14 (−436 to 404)	0.28
CD34⁺/CD133⁺	−28 (−203 to 235)	11 (−203 to 291)	−57 (−266 to 90)†	0.06	−28 (−244 to 261)	−45 (−216 to 289)	−4 (−260 to 230)	0.59
CD34⁺/CXCR4⁺	−17 (−323 to 392)	24 (−209 to 529)†	−115 (−406 to 79)†	0.001	−28 (−419 to 340)	114 (−365 to 455)	−195 (−444 to 181)	0.09
Leukocyte counts, ×10³ cells/µL								
Total white blood cells	0.1 (−0.5 to 0.8)	0.15 (−0.5 to 0.8)	0 (−0.6 to 0.7)	0.31	0.2 (−0.4 to 0.8)	0.3 (−0.4 to 0.9)	0.05 (−0.7 to 0.8)	0.31
Mononuclear cells	0 (−0.2 to 0.1)	0 (−0.2 to 0.1)	0 (−0.1 to 0.2)	0.37	0 (−0.2 to 0.1)	−0.1 (−0.2 to 0.1)	0 (−0.2 to 0.2)	0.11
Hemoglobin, g/dL	0.3 (−0.3 to 0.7)†	0.25 (−0.3 to 0.7)†	0.3 (−0.3 to 0.7)†	0.87	0.2 (−0.1 to 0.8)	0.2 (−0.1 to 0.8)†	0.25 (−0.2 to 0.7)	0.22
Chemokines, pg/mL								
VEGF	7.86 (−0.7 to 19)†	7.33 (−1.1 to 16.9)†	8.93 (0.3 to 24.4)†	0.38	...	...	...	...
SDF-1α	−51 (−298 to 175)†	−72 (−364 to 139)†	11 (−177 to 276)	0.01	...	...	...	...

Median percentage change (25th–75th percentile) is shown. SDF-1α indicates stromal-derived factor-1α; and VEGF, vascular endothelial growth factor.

*P value compares the change in those with and without ischemia.

†Indicates P<0.05 for comparing levels before and after stress testing.

contributes to collateral formation in ischemic tissues.[1–3,27,28] This is also compatible with observations of improvement in ischemic symptoms in patients with chronic ischemia treated with CD34⁺ PC cell therapy.[14]

Although a bout of exercise or regular exercise training has been shown to increase circulating PCs, changes in circulating PC counts in the setting of acute myocardial ischemia have not been previously well studied.[29–33] Most of the previous studies have assessed PCs ≈24 hours to a few days or weeks after participation in regular exercise programs and have shown a significant increase in PCs.[31,34–38] Only one study of 34 patients showed a transient increase in CD34⁺/KDR⁺ (kinase insert domain-containing receptor) cells between 2 and 48 hours in those with, but not in those without, ST depression with exercise.[39] Other studies that have assessed levels of PCs after exercise recruited a heterogeneous population, including healthy subjects, patients with peripheral vascular disease, or patients with CAD.[31,40–43] Higher PCs were reported after 4 weeks of ischemia, provoking exercise program in patients with peripheral vascular disease or CAD.[37] Similarly, higher PCs were reported in an experimental model of limb ischemia,[36] in patients with chronic microvascular myocardial ischemia,[38] and 8 days after an acute myocardial infarction.[20,44] However, these studies are not comparable to our study because of differences in exercise protocols and study populations, lack of information about myocardial ischemia, and, most important, differences in the timing of PC assessments. Our investigation included a significantly larger population with well-defined stable CAD, used state-of-the-art radioisotope imaging for quantitation of myocardial ischemia, and used 2 control populations (patients without ischemia and those with flow mismatch after pharmacological stress testing).

Exercise is associated with increased myocardial oxygen demand that can lead to the development of myocardial ischemia in patients with obstructive CAD. In contrast, pharmacological stress testing causes coronary vasodilatation, leading to flow mismatch in the region supplied by the obstructed coronary artery, without precipitation of ischemia. The observed ≈18% reduction in circulating CD34⁺/CXCR4⁺ PCs 45 minutes after exercise-induced ischemia, when PC levels increased by ≈15% in those without exercise-induced ischemia, can be explained by homing of this subset to the ischemic myocardium, a process observed in experimental models.[21] This hypothesis is supported by findings of avid homing of intracoronary injected labeled CD34⁺ cells into the viable but ischemic peri-infarct tissue in patients receiving bone marrow cells after myocardial infarction,[45–47] as well as a higher myocardial engraftment in those with microvascular ischemia detected using coronary flow reserve.[47]

Ischemia can induce significant mobilization of PCs from their vascular niches in the bone marrow into the peripheral blood.[18,34,36,37,41,48,49] PCs are maintained in the vascular
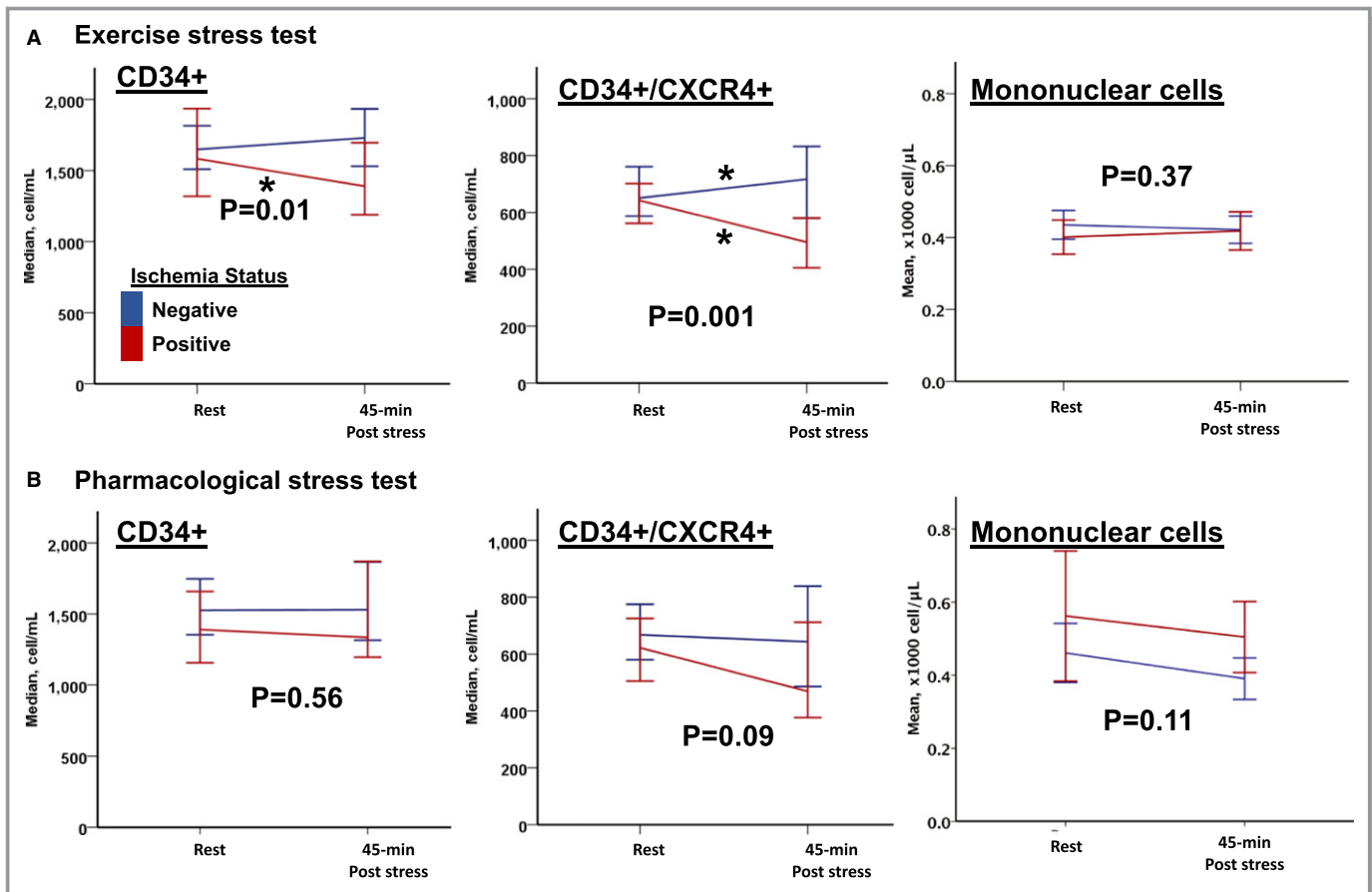
Figure 1. Progenitor cell response to exercise stress test (A) and pharmacological stress test (B) in patients with and without ischemia. *P* values compare the changes in cells before and after stress testing in those with and without ischemia.

niches in a bound form between the basement membrane and various molecules, including CXCR4, SDF-1α, c-kit, adhesion molecules, and stem cell factor.[34,49,50] Chemokines that promote mobilization and homing of PCs[51] include VEGF, SDF-1α, and matrix metalloproteinases released from ischemic tissues.[19–22] Binding of SDF-1α to its receptor CXCR4 promotes homing of PCs and activates phosphoinositide-3 kinase–dependent chemotaxis, cell migration, and secretion of matrix metalloproteinases and VEGF.[17,18,52–56]

Exercise in our patients with CAD increased VEGF levels, but there was no difference in the magnitude of increase in VEGF levels between those with and without ischemia. Previous studies have reported increases,[18,37,39,40] reduction,[57] or no change in VEGF levels with exercise.[37,58] However, exercise in those with myocardial ischemia[39] or in those with severe obstructive peripheral arterial disease[37] resulted in a significant increase in VEGF levels, likely through induction of hypoxia-inducible factor 1.[59] Although this

Table 3. Linear Regression Model for the Association Between Changes in Progenitor Cell Counts in Patients With and Without Myocardial Ischemia During Exercise and Pharmacological Stress Testing

Variable	Change in CD34^{+} Cells/mL (95% CI)	*P* Value	Change in CD34^{+}/CD133^{+} Cells/mL (95% CI)	*P* Value	Change in CD34^{+}/CXCR4^{+} Cells/mL (95% CI)	*P* Value
Positive vs negative exercise test result						
Unadjusted	−310 (−584 to −37)	0.03	−57 (−206 to 92)	0.45	−346 (−542 to −150)	0.001
Adjusted	−363 (−635 to −92)	0.01	−87 (−236 to 61)	0.25	−374 (−572 to −175)	0.0003
Positive vs negative pharmacological test result						
Unadjusted	−89 (−464 to 287)	0.64	−100 (−342 to 143)	0.42	−297 (−618 to 24)	0.07
Adjusted	−120 (−494 to 254)	0.53	−116 (−360 to 128)	0.35	−300 (−628 to 27)	0.07

Adjusted for age, sex, body mass index, race, history of myocardial infarction, hypertension, diabetes mellitus, and renal function. CI indicates confidence interval.

Table 4. Spearman Rank Correlations Between Progenitor Cells and Chemokine Levels

| Variable | Exercise Stress | | | |
| | Change in VEGF | | Change in SDF-1α | |
	Negative r (P Value)	Positive r (P Value)	Negative r (P Value)	Positive r (P Value)
Change in PC counts during physical stress				
CD34$^+$	0.03 (0.72)	−0.09 (0.42)	−0.06 (0.47)	−0.24 (0.03)
CD34$^+$/CD133$^+$	−0.01 (0.93)	0.05 (0.68)	−0.04 (0.63)	−0.22 (0.05)
CD34$^+$/CXCR4$^+$	0.01 (0.93)	0.02 (0.88)	−0.03 (0.7)	−0.06 (0.61)

PC indicates progenitor cell; SDF-1α, stromal-derived factor-1α; and VEGF, vascular endothelial growth factor.

increase in VEGF with exercise was associated with a simultaneous increase in circulating PCs in nonischemic patients, circulating PC levels were reduced in ischemic patients, presumably because, despite mobilization from the bone marrow by VEGF, they were homing to the ischemic myocardium.

We also found a significant inverse correlation between the change in SDF-1α levels and the change in PCs in those who developed ischemia during exercise stress testing. SDF-1α is expressed in atherosclerotic plaques and ischemic tissue,[49] and in the presence of a high SDF-1α gradient between the ischemic tissue, peripheral blood, and the bone marrow, PCs mobilize and home into the ischemic tissue.[27,60] Thus, plasma levels of SDF-1α may reflect higher consumption of PCs by the injured plaque/myocardium.[61] Previous studies have reported decrease,[58,57] no change,[62,63] or increase in SDF-1α level with exercise.[48]

The prognostic and therapeutic role of CD34$^+$ cells is increasingly being recognized.[25,26,64] Preclinical studies show that CD34$^+$ cells stimulate neovascularization in ischemic tissues and increase capillary density and improve cardiac function in acute and chronic myocardial or peripheral arterial ischemia, effects that are largely likely attributable to their paracrine actions.[3,9,48,65–67] Several therapeutic cell therapy trials with CD34$^+$ cells have noted improvement in ischemia and function.[11,13–15,68]

Strengths and Limitations

This is the largest and most comprehensive evaluation of the in vivo effects of transient myocardial ischemia on circulating PCs, where myocardial ischemia and PC levels were measured using state-of-the-art and reproducible techniques. Limitations include lack of functional PC assays and an extended timing of blood sampling. Also, we have not performed radiolabeled imaging to demonstrate increased PC homing. However, previous studies have shown that radiolabeled CD34$^+$ cells, homed to areas of peri-infarct ischemia[46] and ischemic exercise in patients with atherosclerosis, resulted in an increased rate of PC incorporation into vascular structures because of upregulation of homing factors, like CXCR4.[37]

The decrease in CD34$^+$/CXCR4$^+$ cell counts was not significant in those who had a positive pharmacological stress test result. Although we have used the pharmacological stress group as a control population, the lack of changes within this subset may be because of differences in risk factors and other unmeasured differences that influence regenerative capacity. However, the baseline PC counts were similar in those who underwent exercise compared with those undergoing pharmacological stress testing.

Figure 2. Correlation between change in stromal-derived factor-1α (SDF-1α) tertiles and median change in CD34$^+$ cells.

Conclusion

Exercise-induced transient myocardial ischemia is associated with a significant decrease in circulating CD34$^+$/CXCR4$^+$ cell counts, likely because of their SDF-1α–mediated homing to the ischemic myocardium.
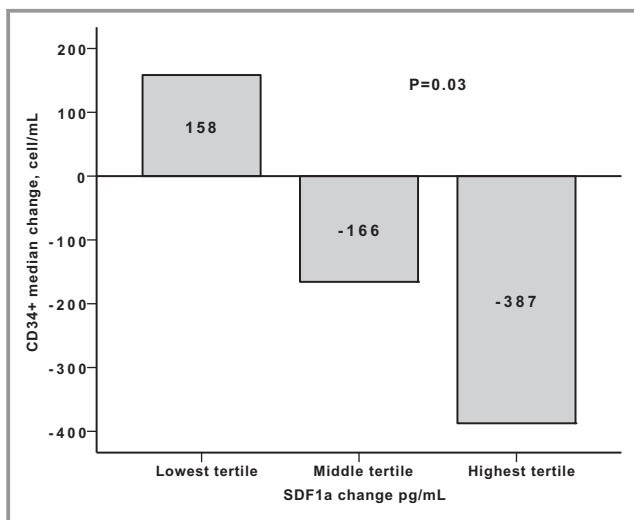
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Disclosures

None.

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