Myocardial Ischemia and Mobilization of Circulating Progenitor Cells

Muhammad Hammadah, Emory University
Ayman Samman Tahhan, Emory University
Ibhar Al Mheid, Emory University
Kobina Wilmot, Emory University
Ronnie Ramadan, Emory University
Bryan R. Kindya, Emory University
Heval M. Kelli, Emory University
Wesley T. O'Neal, Emory University
Pratik Sandesara, Emory University
Samaah Sullivan, Emory University

Only first 10 authors above; see publication for full author list.

Journal Title: Journal of the American Heart Association
Volume: Volume 7, Number 4
Publisher: Wiley Open Access: Creative Commons Attribution Non-Commercial | 2018-02-20
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1161/JAHA.117.007504
Permanent URL: https://pid.emory.edu/ark:/25593/s8q6b

Final published version: http://dx.doi.org/10.1161/JAHA.117.007504

Copyright information:
© 2018 The Authors.
This is an Open Access work distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/).

Accessed April 24, 2020 11:16 PM EDT
Myocardial Ischemia and Mobilization of Circulating Progenitor Cells

Muhammad Hammadah, MD;* Ayman Samman Tahhan, MD;* Ibahil Al Mheid, MD; Kobina Wilmot, MD; Ronnie Ramadan, MD; Bryan R. Kindya, MD; Heval M. Kelli, MD; Wesely T. O’Neal, MD, MPH; Pratik Sandesara, MD; Samaah Sullivan, PhD; Zakaria Almuwaqqat, MD; Malik Obdeen, MD; Naser Abdelhadi, MD; Ayman Alkhoder, MD; Pratik M. Pimple, MD, MPH; Oleksiy Levantsevych, MD; Kareem H. Mohammed, MD; Lei Weng, MPH; Laurence S. Sperling, MD; Amit J. Shah, MD, MSCR; Zakaria Almuwaqqat, MD; Malik Obideen, MD; Lei Weng, MPH; Laurence S. Sperling, MD; Amit J. Shah, MD, MSCR; Zsuzsanna Garamyi, PhD; Mohammad S. Ahmad, MD; Tarek El-Jawahri, MD; Wei Cui, PhD;, Yan V. Sun, PhD; Brad D. Pearce, PhD; Michael Kutner, PhD; Laura Ward, MPH; J. Douglas Bremner, MD; Jianhui Kim, PhD; Edmund K. Waller, MD, PhD; Paolo Raggi, MD; David Sheps, MD, MSPH; Viola Vaccarino, MD, PhD; Arshed A. Quyyumi, MD

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 (14.7% and 25.3%) 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 (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+/CD45med) and nonhematopoietic progenitors.8

From the Division of Cardiology, Department of Medicine (M.H., A.S.T., I.A.M., K.W., R.R., B.R.K., H.M.K., W.T.O., P.S., Z.A., M.O., N.A., A.A., K.H.M., L.S.S., A.J.S., V.V., A.A.Q.), and Department of Psychiatry and Behavioral Sciences (J.D.B.), Emory University School of Medicine, Atlanta, GA; Department of Epidemiology (S.S., P.M.P., O.L., L.W., A.J.S., Y.K.S., B.D.P., V.V.), Department of Biostatistics and Bioinformatics (Y.V.S., M.K., L.W.), and Department of Hematology and Oncology, Winship Cancer Institute (J.K., E.K.W.), Rollins School of Public Health, Emory University, Atlanta, GA; Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Alberta, Canada (P.R.); and Department of Epidemiology, University of Florida, Gainesville, FL (D.S.).

*Dr Hammadah and Dr Samman Tahhan contributed equally to this work.

Correspondence to: Arshed A. Quyyumi, MD, Department of Cardiology, Emory University School of Medicine, Emory University, 1462 Clifton Rd NE, Ste 507, Atlanta, GA 30322. E-mail: aqquyumi@emory.edu

Received August 28, 2017; accepted December 13, 2017.

© 2018 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
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.

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-1α from the ischemic myocardium leads to homing of these cells to the ischemic myocardium in proportion to the magnitude of ischemia. 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/
Myocardial Ischemia and CD34+/CXCR4+ Cells

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 midlevel modifier 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.

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 r=−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+/CXCR4-negative 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>
<thead>
<tr>
<th>Variable</th>
<th>Exercise Stress</th>
<th>Pharmacological Stress</th>
<th>P Value*</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Subjects (n=245)</td>
<td>Negative for Ischemia (n=168)</td>
<td>Positive for Ischemia (n=77)</td>
<td>Negative for Ischemia (n=111)</td>
</tr>
<tr>
<td>Age, y</td>
<td>62.2±8.6</td>
<td>61.9±8.9</td>
<td>62.9±8</td>
<td>0.47</td>
</tr>
<tr>
<td>Male sex</td>
<td>191 (77.96)</td>
<td>127 (75.6)</td>
<td>64 (83.12)</td>
<td>0.19</td>
</tr>
<tr>
<td>White race</td>
<td>181 (73.88)</td>
<td>127 (75.6)</td>
<td>54 (70.13)</td>
<td>0.37</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.6±5.2</td>
<td>29.5 (5.4)</td>
<td>29.7 (4.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>Hypertension</td>
<td>178 (72.65)</td>
<td>119 (70.83)</td>
<td>59 (76.62)</td>
<td>0.35</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>200 (81.63)</td>
<td>134 (79.76)</td>
<td>66 (85.71)</td>
<td>0.26</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>68 (27.76)</td>
<td>47 (27.98)</td>
<td>21 (27.27)</td>
<td>0.91</td>
</tr>
<tr>
<td>Smoking</td>
<td>139 (56.97)</td>
<td>93 (55.69)</td>
<td>46 (59.74)</td>
<td>0.55</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>91 (37.14)</td>
<td>65 (38.69)</td>
<td>26 (33.77)</td>
<td>0.46</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min per 1.73 m²</td>
<td>82.7±20.8</td>
<td>81.8±20.2</td>
<td>84.8±22</td>
<td>0.34</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>53.9±11.3</td>
<td>54.3±11</td>
<td>52.9±12</td>
<td>0.33</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>222 (90.61)</td>
<td>151 (89.88)</td>
<td>71 (92.21)</td>
<td>0.56</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>121 (49.39)</td>
<td>77 (45.83)</td>
<td>44 (57.14)</td>
<td>0.10</td>
</tr>
<tr>
<td>ARBs</td>
<td>38 (15.51)</td>
<td>28 (16.67)</td>
<td>10 (12.99)</td>
<td>0.46</td>
</tr>
<tr>
<td>β Blocker</td>
<td>172 (70.2)</td>
<td>118 (70.24)</td>
<td>54 (70.13)</td>
<td>0.99</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>89 (36.33)</td>
<td>58 (34.52)</td>
<td>31 (40.26)</td>
<td>0.39</td>
</tr>
<tr>
<td>Statins</td>
<td>215 (87.76)</td>
<td>145 (86.31)</td>
<td>70 (90.91)</td>
<td>0.31</td>
</tr>
<tr>
<td>Leukocyte counts, ×10³ cells/μL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cells</td>
<td>5.9±1.7</td>
<td>6±1.8</td>
<td>5.7±1.5</td>
<td>0.22</td>
</tr>
<tr>
<td>Absolute mononuclear cell count</td>
<td>0.4±0.3</td>
<td>0.4±0.3</td>
<td>0.4±0.2</td>
<td>0.61</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>14±1.5</td>
<td>13.9±1.4</td>
<td>14.2±1.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Resting progenitor cells, median (IQR), cells/μL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34⁺</td>
<td>1596 (1049–2423)</td>
<td>1609 (1110–2430)</td>
<td>1467 (1006–2423)</td>
<td>0.81</td>
</tr>
<tr>
<td>CD34⁺/CD34⁻/CD45⁺/CD34⁻/CD45⁻</td>
<td>674 (360–1033)</td>
<td>699 (366–1045)</td>
<td>673 (333–970)</td>
<td>0.52</td>
</tr>
<tr>
<td>Resting chemokines, median (IQR), pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>52 (37–76)</td>
<td>52 (36–76)</td>
<td>53 (38–76)</td>
<td>0.61</td>
</tr>
<tr>
<td>SDF-1α</td>
<td>1162 (968–1430)</td>
<td>1162 (971–1430)</td>
<td>1169 (946–1449)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise Stress (n=245)</th>
<th>Pharmacological Stress (n=111)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Subjects Negative for Ischemia</td>
<td>Positive for Ischemia</td>
<td></td>
</tr>
<tr>
<td>Progenitor cells, cells/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+ cells</td>
<td>6 (−377 to 362)</td>
<td>54 (−295 to 509)</td>
<td>0.007</td>
</tr>
<tr>
<td>CD34+/CD133+</td>
<td>−28 (−203 to 235)</td>
<td>11 (−203 to 291)</td>
<td>0.06</td>
</tr>
<tr>
<td>CD34+/CXCR4+</td>
<td>−17 (−323 to 392)</td>
<td>24 (−209 to 529)</td>
<td>0.001</td>
</tr>
<tr>
<td>Leukocyte counts, ×10³ cells/µL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total white blood cells</td>
<td>0.1 (−0.5 to 0.8)</td>
<td>0.15 (−0.5 to 0.8)</td>
<td>0.31</td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>0 (−0.2 to 0.1)</td>
<td>0 (−0.2 to 0.1)</td>
<td>0.37</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>0.3 (−0.3 to 0.7)</td>
<td>0.25 (−0.3 to 0.7)</td>
<td>0.87</td>
</tr>
<tr>
<td>Chemokines, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>7.96 (−0.7 to 17)</td>
<td>7.33 (−1.1 to 16.9)</td>
<td>0.38</td>
</tr>
<tr>
<td>SDF-1α</td>
<td>−51 (−298 to 175)</td>
<td>−72 (−364 to 139)</td>
<td>11 (−177 to 276)</td>
</tr>
</tbody>
</table>

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.

Although a bout of exercise or regular exercise training has been shown to increase circulating PCs, changes in ischemic symptoms in patients with chronic ischemia treated with CD34+ PC cell therapy have not been previously well studied. Most of the previous studies have assessed PCs 24 hours to a few days or weeks after exercise. Only one study in humans has assessed levels of PCs after exercise. Patients showed a transient increase in CD34+ CD133+ and CD34+ KDR+ (kinase insert domain-containing receptor) cells between 2 and 4 weeks after exercise. Patients with peripheral vascular disease or CAD show the most pronounced increases in PCs after exercise. Patients with CAD and peripheral vascular disease or CAD show the most pronounced increases in PCs after exercise. 33 Most of the previous studies included patients with chronic ischemia treated with CD34+ PC cell therapy. 14

This is also compatible with collateral formation in ischemic tissues. 13,17,28
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 PCs51 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.18,37,39,40 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 ischemia39 or in those with severe obstructive peripheral arterial disease37 resulted in a significant increase in VEGF levels, likely through induction of hypoxia-inducible factor 1.59 Although this

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.

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

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

Adjusted for age, sex, body mass index, race, history of myocardial infarction, hypertension, diabetes mellitus, and renal function. CI indicates confidence interval.
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, 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. Thus, plasma levels of SDF-1α may reflect higher consumption of PCs by the injured plaque/myocardium. Previous studies have reported decrease, no change, or increase in SDF-1α level with exercise.

The prognostic and therapeutic role of CD34+ cells is increasingly being recognized. 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. Several therapeutic cell therapy trials with CD34+ cells have noted improvement in ischemia and function.

**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 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.

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.

**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.

---

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change in VEGF</th>
<th>Change in SDF-1α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative r (P Value)</td>
<td>Positive r (P Value)</td>
</tr>
<tr>
<td>Change in PC counts during physical stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+</td>
<td>0.03 (0.72)</td>
<td>-0.09 (0.42)</td>
</tr>
<tr>
<td>CD34+/CD133+</td>
<td>-0.01 (0.93)</td>
<td>0.05 (0.68)</td>
</tr>
<tr>
<td>CD34+/CXCR4+</td>
<td>0.01 (0.93)</td>
<td>0.02 (0.88)</td>
</tr>
</tbody>
</table>

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

Vaccaro and Quyyumi report research support from the National Institutes of Health (NIH). Ernest V. Garcia receives royalties from the sale of the Emory Cardiac Toolbox, used for some analyses in this study. None of the other authors report a conflict of interest relevant to this article. This work was supported by the NIH (P01 HL101398, P20HL113451-01, P01HL086773-06A1, R56HL126558-01, R01 HL109413, R01HL109413-02S1, UL1TR000454, KL2TR000455, K24HL077506, and K24 MH076955). Kelli and Tahhan have been supported by the Abraham J. and Phyllis Katz Foundation. O’Neal has been supported by American Heart Association grant F32HL134290. Kelli and Sullivan have been supported by National Heart, Lung, and Blood Institute T32 grant THL130025A. The sponsors of this study had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the article.

Disclosures
None.

References

Myocardial Ischemia and CD34+/CXCR4+ Cells

Hammadah et al


