Association of Colony-Forming Units With Coronary Artery and Abdominal Aortic Calcification

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Background—Certain bone marrow–derived cell populations, called endothelial progenitor cells, have been reported to possess angiogenic activity. Experimental data suggest that depletion of these angiogenic cell populations may promote atherogenesis, but limited data are available on their relation to subclinical atherosclerotic cardiovascular disease in humans.

Methods and Results—We studied 889 participants of the Framingham Heart Study who were free of clinically apparent cardiovascular disease (mean age, 65 years; 55% women). Participants underwent endothelial progenitor cell phenotyping with an early-outgrowth colony-forming unit assay and cell surface markers. Participants also underwent noncontrast multidetector computed tomography to assess the presence of subclinical atherosclerosis, as reflected by the burden of coronary artery calcification and abdominal aortic calcification. Across decreasing tertiles of colony-forming units, there was a progressive increase in median coronary artery calcification and abdominal aortic calcification scores. In multivariable analyses adjusting for traditional cardiovascular risk factors, each 1-SD increase in colony-forming units was associated with a \( \approx 16\% \) decrease in coronary artery calcification \( (P=0.02) \) and 17% decrease in abdominal aortic calcification \( (P=0.03) \). In contrast, neither CD34\(^+\)/KDR\(^+\) nor CD34\(^+\) variation was associated with significant differences in coronary or aortic calcification.

Conclusions—In this large, community-based sample of men and women, lower colony-forming unit number was associated with a higher burden of subclinical atherosclerosis in the coronary arteries and aorta. Decreased angiogenic potential could contribute to the development of atherosclerosis in humans. (Circulation. 2010;122:1176-1182.)

Key Words: angiogenesis ■ atherosclerosis ■ epidemiology ■ risk factors ■ vasculature

Certain peripherally circulating cell populations are believed to have endothelial reparative and angiogenic properties and thus have been called endothelial progenitor cells (EPCs).\(^1\) Experimental studies indicate that EPCs, broadly defined, are capable of promoting neovascularization in the setting of arterial ischemia\(^2,\(^3\) and reendothelialization after mechanical arterial injury.\(^4\) Furthermore, EPC-related traits have been associated with cardiovascular risk factors in some clinical studies,\(^5-\(^8\) and data from selected samples suggest that decreased EPC number is related to an increased risk for adverse cardiovascular outcomes.\(^9,\(^10\) Thus, it has been hypothesized that chronic depletion of EPCs could contribute to the development of atherosclerosis.

Clinical Perspective on p 1182

Prior investigations of the association between circulating angiogenic cell phenotypes and subclinical vascular disease have been relatively small. In studies involving <100 subjects each, a lower quantity of various EPC-related phenotypes has been associated with abnormal flow-mediated dilation\(^6,\(^11-\(^14\) and greater arterial stiffness.\(^15\) However, studies that have directly measured the presence and burden of...
subclinical atherosclerosis have not consistently demonstrated an association with EPC traits.7,8,11,16–22 Data are particularly conflicting with respect to measures of advanced subclinical atherosclerosis such as coronary artery calcification (CAC).5,10 If EPCs are critical for both maintaining endothelial integrity and facilitating arterial repair, chronically low EPC supply could predispose to atherosclerotic plaque formation. The presence of calcified plaque in the coronaries23–25 and the aorta26,27 is considered a reliable marker of atherosclerosis and strongly predicts future cardiovascular events.23 Thus, an association of EPCs with arterial calcification would support the hypothesis that EPC depletion contributes to the progression from subclinical endothelial dysfunction to cardiovascular disease in humans. We assessed EPC-related traits in a large community-based sample and investigated whether low EPC quantity was associated with measures of coronary and aortic arterial calcification detected by multidetector computed tomography (CT).

Methods

Study Sample

In 1948, the Framingham Heart Study enrolled 5209 residents of Framingham, Mass, in a longitudinal cohort study designed to identify risk factors for cardiovascular disease.28 In 1971, a total of 5124 offspring of the original cohort (and their spouses) were enrolled in the Framingham Offspring Study.29 All participants in the offspring study receive routine examinations approximately every 4 years, and a total of 3021 participants attended their eighth examination cycle (2005 through 2008). Of this sample, 997 had phenotyping of EPC-related traits at the eighth examination and had undergone assessment of CAC and abdominal aortic calcification (AAC) by multidetector CT between the seventh and eighth examinations (2002 through 2005). The multidetector CT scans occurred a mean of 2.6±0.9 years before the assessment of EPC phenotypes, which occurred during the eighth examination visit. The majority (n=889, 89%) of these participants did not have known cardiovascular disease (history of myocardial infarction, coronary insufficiency, stroke, and heart failure) and were eligible for the present analysis. All participants gave informed consent, and the institutional review board of the Boston University School of Medicine approved all study protocols.

Clinical and Risk Factor Assessment

All study participants underwent a standardized medical examination and laboratory assessment of cardiovascular risk factors. Systolic and diastolic blood pressures were the average of 2 physician-measured readings. Body mass index was calculated as weight divided by height squared (kg/m²). Blood was drawn for glucose, total and high-density lipoprotein (HDL) cholesterol, and triglycerides after an overnight fast. Use of medications and cigarette smoking (current smoker within the prior year, past smoker, never smoking) were self-reported. Diabetes mellitus was defined as having fasting blood glucose ≥126 mg/dL or taking medications to treat diabetes mellitus. Alcohol use was defined as ingestion of >14 or >7 alcoholic beverages per week in men or women, respectively. Physical activity was assessed with a physical activity index calculated from the number of hours spent each day at various activity levels weighted according to the estimated oxygen consumption required for each activity.30 Education level was self-reported and assessed as a categorical variable (less than high school completion, high school diploma or equivalent but no college degree, completed college degree or higher). C-reactive protein was assayed with the immunoturbidimetric latex-enhanced high-sensitivity assay (Roche Diagnostics, Indianapolis, Ind).

Assessment of Cell Phenotypes

Fasting blood specimens were collected from participants in the morning between 8 and 9 AM for assaying the following angiogenic cell phenotypes: colony-forming units (CFUs), CD34+ cells, and CD34+/KDR- cells. After the initial centrifugation of each blood specimen, the resulting buffy coat was further processed for cell phenotyping within 4 hours of specimen collection as previously described6–10 with modifications. Specifically, buffy coat samples were diluted to 10.5 ml with PBS (Invitrogen, Carlsbad, Calif) and layered over 5 ml Ficoll (Amersham Pharmacia Biotech, Piscataway, NJ). Each specimen was then centrifuged at 2200 rpm for 15 minutes at 10°C. Peripheral blood mononuclear cells were isolated from the buffy coat with Ficoll density-gradient centrifugation and then processed for CFU assay and flow cytometry.

CFU Assay

Collected peripheral blood mononuclear cells were washed with PBS and then lysed with ACK lysis buffer (Fisher Scientific, Pittsburgh, Pa). Viable mononuclear cells, totaling 5 million per specimen, were then plated in each well of a 6-well fibronectin-coated tissue culture plate (BD Biosciences, San Jose, Calif) in M199/20% FBS and cultured at 37°C/5% CO2. Nonadherent cells were collected after 2 days, and 2 million viable cells in M199/20% FBS were replated in wells of a 24-well fibronectin-coated tissue culture plate (BD Biosciences). After nonadherent cells were cultured for an additional 5 days, the newly formed colonies in each well were counted. Each distinct colony was identified by specific morphological characteristics that have previously been described.6 A single blinded technician performed colony counting and reported counts as the average number of colonies per well across up to 12 wells. In wells in which the colonies were too numerous to count (mean, 4.7 wells from 63 individuals), the number of colonies per well was censored at 300. A single technician (R.P.M.) performed the initial plating of cells and colony counting for all specimens except for a small subset (K.S.C.). Replating of cells was performed by 1 of 2 technicians (R.P.M., R.J.K.). To minimize the effects of operator variation, colony counts were standardized by identity of the replater.

Flow Cytometry

Peripheral blood mononuclear cells were incubated for 15 minutes with FcR blocking agent (Miltenyi Biotec, Bergisch Gladbach, Germany) on ice and then for 25 minutes on ice with anti-KDR PE (R&D Systems, Minneapolis, Minn) and anti-CD34 FITC (BD Biosciences) anti-human antibodies. Samples were washed and then fixed in 2% paraformaldehyde. Expression of the surface markers was evaluated by fluorescence-activated cell sorter analysis; the number of positive cells was quantified with a Becton-Dickinson FacsCalibur flow cytometer, with fluorochrome-matched IgG isotype controls. Red blood cells, platelets, and cell debris were excluded by use of forward- and side-scatter electronic characteristics. The frequency of CD34+ cells was then identified within the neucleated cell gate with population gating. Finally, KDR- events within the CD34 population were analyzed via population gating. CD34+ and CD34+/KDR- cells were quantified with FlowJo analysis software (Treestar, Ashland, Ore).31 Quaties of each cell type were reported as a percentage of the total number of gated neucleated cells. All flow analysis plots were reviewed by a blinded investigator (K.S.C.) to ensure consistency.

Assessment of Arterial Calcification

Imaging of the chest and abdomen with an 8-slice multidetector CT (Lightspeed Ultra, General Electric, Milwaukee, Wis) scanner was performed for all participants as previously described.32 Two chest scans and 1 abdominal scan were performed for each participant using a sequential scan protocol with a slice collimation of 8×2.5 mm [120 kVp], 320/400 mA for a cut point of 220-lb body weight, respectively) during a single end-inspiratory breath hold (typical duration, 18 seconds). Image acquisition (330 ms) was prospectively initiated at 50% of the cardiac cycle. For abdominal scanning, 30 contiguous 5-mm-thick slices of the abdomen were...
acquired, covering 150 mm above the level of S1. A calibration phantom (Image Analysis, Lexington, Ky) containing rods of water and 75 and 150 mg/cm² calcium hydroxyapatite was placed under-neath each participant.

A trained technician performed calcium measurements for each study using an offline workstation (Acqaurus, Terarecon, San Matteo, Calif). The method for scoring CAC has been described previously, along with excellent intrarater and interrater reproducibility for CAC measurements.32 A calcified lesion in either the coronary arteries or the aorta was defined as an area of ≥3 connected pixels with CT attenuation >130 Hounsfield units using 3-dimensional connectivity criteria. A score for AAC (from the abdominal scan) and CAC (from each of the 2 chest scans) was calculated by multiplying the area of a calcified lesion by a CT attenuation score weighted on the basis of the maximal CT attenuation (Hounsfield units) within a lesion. Because the Agatston score was originally developed for electron-beam CT,13 we applied a modified Agatston score algorithm to our multidetector CT scan protocol to score for CAC and for AAC, as has been done in numerous prior studies.32,34

**Statistical Analyses**

Because of highly skewed distributions, natural logarithmic-transformed values were applied for triglycerides, CD34⁺/KDR⁺, and CD34⁺. Square root-transformed values were used for CFU after standardization by technician replater. We used natural logarithmic-transformed values for CAC and AAC; log(CAC+1) and log(AAC+1), respectively, were used in analyses to account for zero values.

Associations of each measure of arterial calcium (CAC and AAC) with each cell phenotype (CFU, CD34⁺, and CD34⁺/KDR⁺) were characterized with linear regression models adjusting for age and sex as independent covariates. We also analyzed the association of CFU quantity with increasing number of traditional cardiovascular risk factors, defined as older age (>45 years for men, >55 years for women), male sex, hypertension, hypercholesterolemia, diabetes mellitus, current smoker, and obesity. Multivariable regression models adjusted for clinical covariates known to correlate with measures of subclinical atherosclerosis have been evaluated in numerous prior studies.32,34 However, data on the relation of EPCs to endothelial function and arterial compliance.6,11,12,14,15,36 However, data on the relation of EPCs to endothelial function and arterial compliance.6,11,12,14,15,36

In secondary analyses, we repeated multivariable analyses, including further adjustment for C-reactive protein. We also tested for effect modification by age or sex using multiplicative interaction terms in the multivariable models. All analyses were performed with SAS statistical software (GENMOD procedure), version 9.1.3 (SAS Institute Inc, Cary, NC). A 2-tailed value of P<0.05 was considered significant.

**Results**

Clinical characteristics of the study sample are shown in Table 1. The mean age was 65 years, and 55% were women. The median values of CFU number, CD34⁺, and CD34⁺/KDR⁺ were 41 (interquartile range, 20 to 62), 0.077% (interquartile range, 0.049% to 0.105%), and 0.003% (interquartile range, 0.002% to 0.005%), respectively, in the total sample. CFU quantity was not correlated with log CD34⁺ (age- and sex-adjusted Pearson correlation coefficient, r=0.06, P=0.12) or log CD34⁺/KDR⁺ (r=0.05, P=0.20). Log CD34⁺ and log CD34⁺/KDR⁺ were moderately correlated (r=0.26, P<0.001). Estimated sibling correlations were 0.124 for log (CAC+1) and 0.236 for log (AAC+1). Individuals with lower CFU had higher CAC and AAC scores. Median CAC scores were 15.0, 22.2, and 34.7 across decreasing CFU tertiles. Corresponding AAC scores were 274.1, 472.0, and 498.1 Agatston units. In this sample, lower CFU number was not associated with increasing number of traditional cardiovascular risk factors.

The results of the multivariable regression analyses are shown in Table 2. There were significant inverse relations of CFU with both CAC and AAC after adjustment for age, sex, and conventional cardiovascular risk factors and accounting for between-sibling correlations. A 1-SD increment in the CFU variable was associated with a −0.177 decrease in log CAC (95% confidence intervals [CI], −0.320 to −0.034), which corresponds to an approximately 16% lower CAC score. Each 1-SD increment in CFU was also associated with a −0.185 decrease in log AAC (95% CI, −0.356 to −0.013), corresponding to an approximately 17% lower AAC score. In contrast, neither CD34⁺ nor CD34⁺/KDR⁺ was associated with CAC or AAC in multivariable analyses (Table 2).

In secondary analyses, the associations of CFU with CAC and AAC were unchanged in multivariable analyses that additionally adjusted for C-reactive protein (P=0.01 and P=0.04 for associations of CFU with CAC and AAC, respectively). We performed analyses testing for effect modification by age and sex. For AAC, there was a statistically significant interaction of age and CFU (P=0.004), with individuals <65 years of age having a stronger association between CAC and AAC (β=−0.365; 95% CI, −0.634 to −0.095) than those ≥65 years of age (β=0.097; 95% CI, −0.096 to 0.290). In contrast, the interaction between age and CFU was nonsignificant for CAC (P=0.18; younger individuals: β=−0.240 [95% CI, −0.429 to −0.050]; older individuals: β=−0.054 [95%, −0.277 to 0.170]). There was no evidence of a sex interaction for the association of CFU with either measure of arterial calcification.

**Discussion**

In a large community-based sample of predominantly healthy men and women, reduced CFU quantity was associated with greater subclinical coronary and aortic calcification. This association was present even after adjustment for cardiovascular risk factors. These findings are consistent with the hypothesis that reduced angiogenic potential could contribute to the development of atherosclerosis.

On the basis of experimental data, it has been proposed that long-term depletion of circulating angiogenic cells, typically referred to as EPCs, leads to an impaired capacity for endothelial repair. Using either culture-based assays or flow cytometry, clinical studies in selected samples have related EPC traits to endothelial function and arterial compliance.5,11,12,14,15,36 However, data on the relation of EPCs with measures of subclinical atherosclerosis have been mixed. In one of the largest of the prior studies, which included 137 middle-aged men and women, Fadini and colleagues17 reported an association of carotid intima-media thickness with CD34⁺/KDR⁺ but not CD34⁺ cell populations. Other studies, however, have found no association between carotid intima-media thickness and CD34⁺/KDR⁺.11,16 Coronary calcification generally re-
reflects the presence of more advanced subclinical atherosclerosis and is a strong predictor of future cardiovascular events.23–27 In a sample of 90 healthy men, Bielak and colleagues8 reported on the inverse relation of CAC with CD34+ cells but did not examine its relationship with other EPC phenotypes. In contrast, Hughes and colleagues19 found no association between CD34+ cells and CAC among 117 European and South Asian men.

Table 1. Sample Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total Sample</th>
<th>First CFU Tertile</th>
<th>Second CFU Tertile</th>
<th>Third CFU Tertile</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>889</td>
<td>258</td>
<td>257</td>
<td>258</td>
</tr>
<tr>
<td>Age, y</td>
<td>65±9</td>
<td>66±9</td>
<td>65±8</td>
<td>64±9</td>
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<tr>
<td>Women, %</td>
<td>55</td>
<td>61</td>
<td>56</td>
<td>47</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.4±5.3</td>
<td>28.0±5.1</td>
<td>29.2±5.8</td>
<td>28.2±5.0</td>
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<td>SBP, mm Hg</td>
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<td>128±17</td>
<td>129±17</td>
<td>127±15</td>
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<tr>
<td>DBP, mm Hg</td>
<td>74±10</td>
<td>74±10</td>
<td>75±10</td>
<td>75±9</td>
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<tr>
<td>Hypertension, %</td>
<td>58</td>
<td>58</td>
<td>65</td>
<td>53</td>
</tr>
<tr>
<td>Hypertension medications, %</td>
<td>48</td>
<td>47</td>
<td>54</td>
<td>45</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>188±36</td>
<td>181±35</td>
<td>189±38</td>
<td>193±32</td>
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<td>HDL cholesterol, mg/dL</td>
<td>57±17</td>
<td>58±17</td>
<td>58±18</td>
<td>56±16</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>3.5±1.0</td>
<td>3.5±1.0</td>
<td>3.5±1.0</td>
<td>3.5±1.0</td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>101 (67–135)</td>
<td>100 (94–107)</td>
<td>102 (73–146)</td>
<td>97 (71–133)</td>
</tr>
<tr>
<td>Cholesterol medications, %</td>
<td>46</td>
<td>42</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>105±21</td>
<td>104±20</td>
<td>105±18</td>
<td>106±27</td>
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<td>Diabetes mellitus, %</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
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<td>Current</td>
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<td>Past</td>
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<td>62</td>
<td>60</td>
</tr>
<tr>
<td>Never</td>
<td>35</td>
<td>36</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>Alcohol use, † %</td>
<td>16</td>
<td>16</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Physical activity index</td>
<td>35.5±5.6</td>
<td>35.7±5.7</td>
<td>35.5±5.7</td>
<td>35.6±5.5</td>
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<tr>
<td>Education level, %</td>
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<td>Less than high school</td>
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<td>2</td>
<td>3</td>
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<tr>
<td>High school diploma</td>
<td>56</td>
<td>54</td>
<td>58</td>
<td>55</td>
</tr>
<tr>
<td>College degree</td>
<td>42</td>
<td>44</td>
<td>39</td>
<td>42</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.
*Values are presented as median (interquartile range) for non-normally distributed variables. All other values are presented as means±SD or percentages.
†Defined as >14 drinks per week for men or >7 drinks per week for women.

Table 2. Results of Linear Regression Models Assessing the Relationship of CT Measures of Calcification With CFU, CD34+, and CD34+/KDR+ Cell Phenotypes

<table>
<thead>
<tr>
<th></th>
<th>Age and Sex Adjusted</th>
<th>Multivariable Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>CAC (as a dependent variable)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFU</td>
<td>−0.155 (−0.303, −0.007)</td>
<td>0.04</td>
</tr>
<tr>
<td>CD34+</td>
<td>0.014 (−0.294, 0.322)</td>
<td>0.93</td>
</tr>
<tr>
<td>CD34+/KDR+</td>
<td>0.020 (−0.177, 0.217)</td>
<td>0.84</td>
</tr>
<tr>
<td>AAC (as a dependent variable)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFU</td>
<td>−0.160 (−0.328, 0.009)</td>
<td>0.06</td>
</tr>
<tr>
<td>CD34+</td>
<td>0.336 (−0.031, 0.702)</td>
<td>0.07</td>
</tr>
<tr>
<td>CD34+/KDR+</td>
<td>−0.007 (−0.227, 0.213)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Regression coefficients represent change in the dependent variable per 1-SD change in square-root CFU, log CD34+, and log CD34+/KDR+ in analyses that accounted for correlated sibling data using generalized estimating equations with exchangeable correlation structure.
*Covariates adjusted for in the multivariable model include age, sex, body mass index, ratio of total to HDL cholesterol, log triglycerides, smoking status, systolic blood pressure, taking medication for hypertension, taking medication for hypercholesterolemia, diabetes mellitus, alcohol use, education level, and physical activity.
To the best of our knowledge, the present study is the largest clinical investigation involving EPC-related traits. Furthermore, our investigation focused on ambulatory individuals without acute illnesses that could lead to mobilization of proangiogenic cells. We observed a significant association of lower CFU with higher CAC and AAC scores. In contrast, we did not observe an association between CD34⁺ or CD34⁺/KDR⁺ and arterial calcification. These apparently discordant findings are consistent with experimental studies suggesting that CFU is a cell-based phenotype distinct from CD34⁺ and CD34⁺/KDR⁺ with respect to both character and function.1,39

CFUs are made up of hematopoietic cells that produce large quantities of angiogenic cytokines and enhance assays of vessel formation both in vitro and in vivo.⁴⁰ The formation of CFUs appears to be dependent on lineage-restricted cell populations, predominantly monocytes and T cells.⁴¹ The dependence of CFU formation on monocyte populations may have particular relevance for their observed association with CAC and AAC. In experimental models, certain monocyte subsets have been observed to promote antiinflammatory rather than proinflammatory activity, in addition to angiogenesis and granulation tissue formation.⁴² Monocyte-derived cells have also been associated with remodeling and regression of arterial calcification.⁴³ Thus, because of their affiliation with the monocytic lineage, it is plausible that the CFU phenotype reflects cellular activities that directly affect the progression or regression of atherosclerotic lesions and, in particular, calcific lesions in the setting of advanced subclinical disease. This effect may be more evident in younger individuals, given the higher prevalence and severity of other factors unrelated to angiogenesis in older individuals that promote atherosclerosis and vascular calcification.⁴³

In contrast to CFUs, the CD34⁺ and CD34⁺/KDR⁺ phenotypes are made up largely of hematopoietic stem/progenitor cells and a smaller population of cells with late-outgrowth endothelial colony formation. Accordingly, prior studies have also shown poor correlation between CD34⁺-related phenotypes and CFUs (which can form in the absence of CD34⁺ cells).¹ CD34⁺ and KDR⁺ phenotypes may lack an observed association with arterial calcification for several reasons. First, CD34⁺ and CD34⁺/KDR⁺ cells may not be physiologically active in processes related to vascular calcification. Because arterial calcification denotes the presence of advanced atherosclerotic plaque, it is possible that CD34⁺ and CD34⁺/KDR⁺ quantity reflects a capacity for endothelial repair that is associated with earlier rather than later manifestations of atherosclerosis or alterations in vascular integrity that do not result in calcification. Second, CD34 and KDR cell surface markers, even when used together, are not highly specific for progenitor cells with endothelial repair and regenerative capacity.¹ CD34 is present not only on hematopoietic stem/progenitor cells but also on mature endothelial cells and embryonic cells.⁴⁴,⁴⁵ Although KDR, or vascular endothelial growth factor receptor 2, may identify a population of circulating hematopoietic cells with more specific endothelial characteristics,¹⁰,⁴⁵ some evidence suggests that even CD34⁺/KDR⁺ cells represent primitive hematopoietic rather than more differentiated endothelial progenitors.⁴⁶ Thus, the ability to identify a true association between biologically relevant progenitor cells and vascular calcification is reduced. Finally, the number of circulating cells positive for both CD34 and KDR is very low in ambulatory individuals,⁴⁷ which may limit statistical power for detecting significant effects. This could also account for differences between our results and those observed in higher-risk populations.⁵,⁹

Several limitations of this study merit consideration. Our analyses were cross-sectional; thus, we cannot determine whether low CFU quantity preceded or followed the development of coronary atherosclerosis. It is also possible that associations of CFU with CAC and AAC are related to unmeasured confounding factors that may influence both colony number and the development of atherosclerosis. In particular, extracellular factors could affect cellular characteristics that contribute to the regenerative capacity of EPCs (eg, paracrine function, migration, resistance to stress, and senescence) and, in turn, result in both lower CFU quantity and increased cardiovascular risk. Because we examined several measures of EPC traits and arterial calcification, we cannot exclude the possibility of false-positive findings resulting from multiple comparisons. Nonetheless, our findings are consistent with studies in referral samples relating CFUs to overt cardiovascular events.⁹ Analysis of endothelial function could provide additional information on factors mediating the observed relation of CFU with arterial calcification; however, concurrent assessments of endothelial function were not available for analysis in our study sample.

Despite their recognized angiogenic potential,⁴⁸ CFUs likely are a heterogeneous population of cells with potentially distinct physiological properties.⁴⁹ Similarly, cells identified as CD34⁺ and CD34⁺/KDR⁺ are likely to include multiple cell subtypes, some of which may not exhibit angiogenic activity. Further stratifying CFUs, CD34⁺, and/or CD34⁺/KDR⁺ cells on the basis of additional surface antigens, markers of senescence, and/or migratory capacity could provide additional insights.⁵⁰ Additionally, our sample was predominantly white and of European ancestry, limiting the generalizability of our findings to other racial/ethnic populations.

**Conclusions**

Lower CFU number was associated with greater coronary and aortic calcification, consistent with a higher burden of subclinical atherosclerosis. In the context of prior studies relating CFU quantity to cardiovascular risk factors and adverse cardiovascular events, these findings suggest that CFUs may represent a distinct marker of circulating angiogenic potential that contributes to vascular health and protects against the pathophysiological changes that promote atherosclerosis. Further research is required to investigate the potential mechanisms underlying the association of CFUs with arterial calcification and to determine whether, indeed, interventions that augment CFU quantity could be effective at preventing or treating clinical cardiovascular disease.

**Sources of Funding**

This work was supported in part by the National Heart, Lung, and Blood Institute’s Framingham Heart Study (contract No. N01-HC-
Disclosures
None.

References


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_Circulation_. 2010;122:1176-1182; originally published online September 7, 2010; doi: 10.1161/CIRCULATIONAHA.109.931279

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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