Levels of Circulating Progenitor Cells, Cardiovascular Outcomes and Death

A Meta-Analysis of Prospective Observational Studies

Mauro Rigato, Angelo Avogaro, Gian Paolo Fadini

Rationale: Circulating progenitor cells (CPCs), including endothelial progenitor cells (EPCs) are biologically related to many aspects of cardiovascular disease, as they promote angiogenesis and vascular repair.

Objective: We herein aimed to meta-analyze studies reporting the prognostic role of the CPC/EPC measure on cardiovascular outcomes and death.

Methods and Results: We screened the English-language literature for longitudinal studies reporting the association between baseline CPC/EPC levels, future cardiovascular events, and death. We retrieved 28 studies, 21 of which contained poolable data and entered the meta-analysis, for a total of 4155 patients, mostly with a high baseline cardiovascular risk. Sixty percent of the studies met at least 11 of 16 items of quality assessment. Overall, reduced CPC/EPC levels were associated with a 2-fold increased risk of future cardiovascular events and cardiovascular death. The most predictive phenotype was CD34+CD133+: low versus high levels predicted overall, reduced CPC/EPC levels were associated with a 2-fold increased risk of future cardiovascular events and cardiovascular death. The most predictive phenotype was CD34+CD133+: low versus high levels predicted

Conclusions: This meta-analysis shows that a reduction in the levels of circulating cells putatively provided with vasculoregenerative properties represents a risk factor for adverse cardiovascular outcomes and death. (Circ Res. 2016;118:1930-1939. DOI: 10.1161/CIRCRESAHA.116.308366.)

Key Words: endothelial progenitor cells ■ epidemiology ■ prevention & control ■ regeneration ■ stem cells

Levels of Circulating Progenitor Cells, Cardiovascular Outcomes and Death

Prediction of future cardiovascular events (CVEs) and death by combinations of traditional risk factors yields inaccurate risk estimates, despite advancements in statistical methods and modeling.1 As a significant proportion of CVE occurs in subjects stratified into low-intermediate risk categories, the sake for cardiovascular risk biomarkers has attracted great interest.2,3 An ideal cardiovascular risk biomarker should be biologically related to disease pathophysiology, easily measurable, associated with prevalent and incident cardiovascular disease (CVD) in different populations, able to improve risk stratification, routinely available in clinical practice, and able to drive clinical decisions.4,5

Circulating progenitor cells (CPCs) are immature bone marrow (BM)–derived cells, mostly of hematopoietic origin, which have been associated with several aspects of CVD, from diagnosis to therapy.5 In clinical studies, CPCs are generally defined by flow cytometry based on the surface expression of the hematopoietic stem cell markers CD34 and CD133. CPCs include phenotypes with vascular endothelial specification, usually called endothelial progenitor cells (EPCs). EPCs account for ≤15% of CPCs and are characterized by the expression of endothelial markers (mostly the type 2 vascular endothelial growth factor receptor KDR).5,6

In animal models, BM-derived progenitor cells contribute to cardiovascular homeostasis by stimulating endothelial repair and angiogenesis through physical integration into the vasculature and paracrine activity.7 In humans, the levels of circulating CPCs and EPCs are reduced in the presence of classical cardiovascular risk factors and established CVD, such as atherosclerosis in the coronary, peripheral, and cerebrovascular district.8 Such reduction represents a putative mechanism of impaired vascular homeostasis and a risk factor for future CVEs. In turn, acute CVEs with ischemia (eg, myocardial infarction or stroke) trigger mobilization of CPCs/EPCs from the BM to peripheral blood,9 possibly as an attempt to provide regenerative cells on request. Failure to do so, is associated with a poorer prognosis.5

Definitions and pathophysiological implications of CPCs/ EPCs have been critically revised,10 but the prognostic impact of their quantification in terms of the risk for future CVE and...
death has never been formally reviewed. Herein, we report
the results of a meta-analysis of studies on the association
between baseline progenitor cell levels and CVE or death. This
overview of available data provides a state-of-the art and criti-
cal information to devise future studies assessing the prognos-
tic capacity of CPCs/EPCs.

Methods

Search for Eligible Papers and Inclusion Criteria
We screened the literature for prospective observational studies reporting
occurrence of CVEs among patients whose levels of CPCs/EPCs were
determined at baseline. Studies were enumeration of cultured endothelial colony-forming cells, often referred to as early EPCs, cir-
culating angiogenic cells, or progenitor cells, were also considered.
Eligible studies had to be reported in the English literature from 1997
to end of February 2016 and could include either patients undergoing
coronary angiography for suspected coronary artery disease or acute
coronary syndrome, or patients admitted for stroke, or patients with-
out acute events but with cardiovascular risk factors. Eligible outcomes
were (1) CVE (defined as myocardial infarction, percutaneous or sur-
gical coronary revascularization, any acute coronary syndrome, heart
failure, stroke, uncontrolled arrhythmia, and cardiovascular death), (2)
cardiovascular death, (3) death from any cause, (4) restenosis (defined
as intrastent luminal loss or stenosis progression accessed by angiogra-
phy), and (5) revascularization. We did not exclude a priori any study
on the basis of methodological standards, sample size, and duration
of follow-up. We searched the MEDLINE database via PubMed up to
February 29, 2016, using the following search terms: (progenitor cells
or CD34+ cells or stem cells) and (cardiovascular events or myocardial
infarction or stroke or angina or tia or failure or hospitalization or reste-
nosis or death or mortality) and (follow-up or follow-up or incident
or incidence). This strategy was complemented by hand searching in
the reference lists of retrieved articles and contact with authors. As 2 of
the authors (A.A. and G.P.F.) of the present article are also authors of
potentially eligible studies, eligibility and risk of bias for such studies
were assessed independently by an author with no secondary interest
(M.R.), as recommended by the Cochrane Collaboration guidelines.11

We identified 695 studies. One duplicate and was excluded. Of
the remaining studies, 666 were irrelevant to this review and were
excluded on the basis of their titles and abstracts. Of the remaining,
7 studies were considered only for descriptive purpose because of
missing data, and 21 were included in meta-analysis, for a total of
4155 patients (Figure 1).

Extraction of Data
We followed the Meta-analysis of Observational Studies in
Epidemiology guidelines for performing and reporting results of ob-
servational studies.12 Two reviewers (M.R. and G.P.F.) independently
extracted data from eligible articles (n=28) using a predefined coding
protocol. Individual item disagreement between the 2 reviewers was
resolved by consensus or consultation with a third author (A.A.). We
extracted information on year of publication, number of patients at
baseline, country, their mean age and percentage of men, the baseline
characteristics curve cut-off, median value, or in relation to their
adjustment variables. When available, we checked the major adverse
coronary event (MACE) breakdown to detect any significant im-
balance in its composition. Studies wherein MACE contained a small
percentage of hard events (myocardial infarction and stroke) were con-
sidered only for more specific outcomes (eg, revascularization).

Quality of Study Reporting
We evaluated the quality of individual study reports according to the
Recommendation for MARKER Prognostic Studies (REMARK)
guidelines for prognostic biomarker studies.13 We extracted details of
16 items related to the purpose of studies, population description, bio-
marker measurement, confounders, outcomes, and analytic choice.
Definitions of each item are given in Online Table I.

Statistical Analysis
We compared the effect of low versus high level of CPCs/EPCs on
all prespecified outcomes. The reported comparisons included risk
estimates onto a standard scale (ie, per 1 SD, per tertile, or per quin-
tile), according to receiver operating characteristics curve cut-off, or
per unit of change in cell count. To allow the comparison on a same
scale, we standardized the risk expressed per unit of change using the
attributable risk approach (Online Table II). The relative risk estimates
of each study and their corresponding SE were transformed to their
natural logarithms to normalize distributions. Because of heterogene-
ity among studies, we used the random-effect meta-analysis using the
inverse variance method. In this approach, the weight given to each
study is the inverse of the variance of the effect estimate. In general, the
larger studies (smaller SE) are given more weight than smaller studies
(larger SE), leading to a reduction of the imprecision of pooled effect
estimate. However, for comparison, we also report summary statistics
obtained using the fixed-effect model, as suggested by Sterne et al.14
For each outcome, we performed an overall meta-analysis and ≤5 sub-
groups analysis: (1) considering only studies wherein patients with-
out acute CVEs were enrolled; (2) considering only studies reporting
adjusted risk estimates onto a standard scale of effect; (3) consider-
only studies wherein patients with acute CVEs were enrolled; (4)
considering only higher quality studies, defined as having a REMARK
score above the median value; (5) considering only studies wherein the
MACE outcome was composed by >50% hard end points (myocardial

<table>
<thead>
<tr>
<th>Nonstandard Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
</tr>
<tr>
<td>CPC</td>
</tr>
<tr>
<td>CVD</td>
</tr>
<tr>
<td>CVE</td>
</tr>
<tr>
<td>EPC</td>
</tr>
<tr>
<td>MACE</td>
</tr>
</tbody>
</table>
Results

Overall Characteristics of Included Studies
The meta-analysis included 21 studies, for a total of 4155 patients (average patients per study=198; median [interquartile range]=154 [121–215]). Characteristics of studies included from meta-analysis are given in the Table. The most important reason whereby studies initially retrieved were finally excluded from meta-analysis was the lack of a poolable risk estimate and impossibility to calculate such estimate from the data provided (Online Table III). Pooled cumulative clinical characteristics of the metaanalyzed patient population are reported in Online Table IV. Four of the 21 studies (n=512 patients, 12.8% of the total population) were performed in patients with acute coronary syndrome, acute myocardial infarction, or stroke.20,25,27,34 For the remaining studies, the underlying disease or condition was elective percutaneous intervention in 7 of 17 studies (n=795 patients; 19.1%),17,19,22,29,31,33 elective coronary angiography for suspected coronary artery disease in 2 of 17 (n=1412 patients; 34.0%),28,32 end-stage renal disease in 4 of 17 studies (n=705 patients; 17.0%); chronic heart failure in 1 of 17 studies (n=156 patients; 3.8%).16 and aortic stenosis in 1 of 17 studies (n=261 patients; 6.3%).35 One study included patients with and without chronic CVD at baseline,21 and 1 study included both healthy subjects and patients with chronic or acute CVD.30 Five of 21 studies considered multiple CPCs/EPCs phenotypes at the same time. Phenotypes most frequently used were CD34+ CPCs (6 studies) and CD34+KDR+ EPCs (12 studies). The outcome most commonly considered was the occurrence of future CVEs (16 studies).

Quality of Included Studies
According to a 16-item evaluation, modified from the REMARK guidelines to fit the purpose of this meta-analysis,13 the median (interquartile range) score was 10 (9–12). Studies had a good quality (>50% of studies) for the following items: prespecified hypothesis, setting, inclusion/exclusion criteria, number of patients at each stage, details on manufacturers and assays for CPCs/EPCs, confounders, hierarchy of outcomes, univariate estimate and adjustment, rationale for group comparisons. Vice versa, quality was overall poor (<50% of studies) for rationale for sample size, description of sample handling, end point validation and masking, handling of missing values (Online Figure I).

Cardiovascular Events
Two studies reporting on CVE were excluded from this analysis because the MACE breakdown indicated an excessive contamination with nonhard events.16,27 The analysis was first conducted for each single-cell phenotype, and then summary statistics for all phenotypes were pooled together. The risk ratio of future CVEs (random-effect model) in patients with low versus high cell count was statistically significant for CD34+CD133+ CPCs (risk ratio [RR], 2.61 [1.44–4.74]), CD34+CD133+KDR+ EPCs (RR, 7.91 [2.65–23.57]), and colony-forming cells (RR, 1.18 [1.00–1.39]), whereas it was not significant for CD34+, CD34+KDR+, and CD133+KDR+ cells. The latter showed significant heterogeneity among studies in the association with future CVEs. When phenotype-specific risk estimates were pooled together, the overall risk ratio indicated that a low CPCs/EPCs count was associated with a significant 97% higher risk of future CVEs (Figure 2).

When the analysis was repeated excluding studies on patients with acute CVD, risk ratio would be significant for CD34+ (2.02 [1.43–2.85]; P<0.001), CD34+CD133+ (2.61 [1.44–4.74]; P<0.002), CD34+KDR+ (1.24 [1.07–1.43]; P=0.003), CD34+CD133+KDR+ (7.91 [2.65–23.57]; P<0.001).

Cardiovascular and All-Cause Mortality
Four studies reported cardiovascular death in relation to baseline CPCs/EPCs. Altogether, they show an association between low versus high cell count and the risk for future cardiovascular death, yielding a pooled risk ratio of 1.87 (95% CI, 1.15–3.02). The corresponding risk ratio according to the fixed-effect model was 1.45 (1.22–1.72). However, multiple studies were available only for CD34+KDR+ cells (n=3), and their association with cardiovascular death was highly heterogeneous, yielding a nonsignificant pooled risk ratio (Figure 3A).

Data on all-cause mortality were available for most phenotypes. The pooled risk ratio of future death in patients with low versus high cell count was statistically significant for CD34+ (RR, 3.40 [1.99–5.83]), CD34+CD133+ CPCs (RR, 2.56 [1.26–5.17]), and CD34+CD133+KDR+ EPCs (RR, 1.36 [1.21–1.53]). Some EPC phenotypes showed paradoxical opposite trend associations with future death from any cause, and a large heterogeneity among studies was found. As a result, the overall summary statistics for all phenotypes was marginally significant with the random-effect model (Figure 3B).

According to the fixed-effect model, the pooled risk ratio for all phenotypes would be 1.37 (1.23–1.52; P<0.001). When the random-effect model analysis excluded studies wherein the risk estimate had to be calculated, the overall statistics was 1.75 (1.23–2.49; P=0.002; Online Figure IIB).

Restenosis and Revascularization
A few studies reported the risk of restenosis after percutaneous intervention and the need for future revascularization in patients with baseline acute or chronic CVD. The association between low versus high CPCs/EPCs levels and restenosis was highly variable according the cellular phenotype, ranging from a significant protection for low CD34+ cells to a significant harm for low CD34+CD133+KDR+ cells or colony-forming cells. The overall statistics showed a trend increased risk of future restenosis in patients with low versus high CPCs/ EPCs, with high and significant heterogeneity (Figure 4A).
### Table. Summary of the Characteristics of Included Studies

<table>
<thead>
<tr>
<th>References</th>
<th>Q %</th>
<th>Country</th>
<th>Follow-Up, mo</th>
<th>Sample Size</th>
<th>Age, y (Mean±SD)</th>
<th>Male, %</th>
<th>Smoker, %</th>
<th>CVD, %</th>
<th>Dyslipidemia, %</th>
<th>Diabetes mellitus, %</th>
<th>Hypertension, %</th>
<th>CKD, %</th>
<th>Statins, %</th>
<th>ACE-i/ ARB, %</th>
<th>Population</th>
<th>Phenotype</th>
<th>Outcomes (Event Rate/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiang et al**</td>
<td>93.3</td>
<td>Taiwan</td>
<td>48</td>
<td>77</td>
<td>68.5±14.5</td>
<td>81.8</td>
<td>48</td>
<td>89.6</td>
<td>59.7</td>
<td>54.5</td>
<td>76.6</td>
<td>59.7</td>
<td>48.5</td>
<td>36.3</td>
<td>Elective PTCA</td>
<td>CD34* KDR+</td>
<td>CVE (8.7%)</td>
</tr>
<tr>
<td>Pelliccia et al**</td>
<td>68.7</td>
<td>Italy</td>
<td>60</td>
<td>155</td>
<td>61.1±10</td>
<td>59.3</td>
<td>26.4</td>
<td>21.9</td>
<td>45.1</td>
<td>14.1</td>
<td>47</td>
<td>n.a.</td>
<td>93.5</td>
<td>41.2</td>
<td>Elective PTCA</td>
<td>CD34* KDR+</td>
<td>CD133*KDR+ All death (2.1%) REV (5.2%)</td>
</tr>
<tr>
<td>Briguori et al**</td>
<td>81.2</td>
<td>Italy</td>
<td>24</td>
<td>136</td>
<td>63.5±13.9</td>
<td>74.2</td>
<td>30.8</td>
<td>36</td>
<td>54.4</td>
<td>42.6</td>
<td>81.6</td>
<td>39.7</td>
<td>88.2</td>
<td>n.a.</td>
<td>Elective PTCA</td>
<td>CFU</td>
<td>RES (18.1%) CVE (21.6%)</td>
</tr>
<tr>
<td>Bonello et al**</td>
<td>68.7</td>
<td>France</td>
<td>6</td>
<td>156</td>
<td>64.7±11.4</td>
<td>75.6</td>
<td>29.5</td>
<td>n.a.</td>
<td>58.3</td>
<td>21.8</td>
<td>57.1</td>
<td>n.a.</td>
<td>69.9</td>
<td>81.4</td>
<td>Elective PTCA</td>
<td>CD34* KDR+</td>
<td>REV (34.6%)</td>
</tr>
<tr>
<td>Schober et al†</td>
<td>50</td>
<td>Germany</td>
<td>8</td>
<td>17</td>
<td>66±8</td>
<td>n.a.</td>
<td>47</td>
<td>47</td>
<td>82.3</td>
<td>29.4</td>
<td>76.4</td>
<td>n.a.</td>
<td>82.3</td>
<td>64.7</td>
<td>Elective PTCA</td>
<td>CD34*</td>
<td>RES (61.7%)</td>
</tr>
<tr>
<td>Wu et al³</td>
<td>62.5</td>
<td>Taiwan</td>
<td>12</td>
<td>130</td>
<td>66±13</td>
<td>36</td>
<td>11</td>
<td>20</td>
<td>18</td>
<td>37</td>
<td>58</td>
<td>100</td>
<td>16</td>
<td>20</td>
<td>PTCA of HD access</td>
<td>CD34* KDR+</td>
<td>CD34<em>CD133</em>KDR+ RES (28%)</td>
</tr>
<tr>
<td>Haine et al**</td>
<td>62.5</td>
<td>Belgium</td>
<td>6</td>
<td>124</td>
<td>61±3.5</td>
<td>77</td>
<td>26</td>
<td>11.2</td>
<td>82</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Elective stenting</td>
<td>CD34* KDR+</td>
<td>RES (n.a.)</td>
</tr>
<tr>
<td>Patel et al³</td>
<td>68.7</td>
<td>United Kingdom</td>
<td>22.4</td>
<td>905</td>
<td>62.6±12.4</td>
<td>63.4</td>
<td>79</td>
<td>26.2</td>
<td>75</td>
<td>31.8</td>
<td>80.6</td>
<td>72.7</td>
<td>n.a.</td>
<td>CVE (5.4%)</td>
<td>CD34*</td>
<td>CVE (5.4%)</td>
<td></td>
</tr>
<tr>
<td>Lee et al²</td>
<td>81.2</td>
<td>Korea</td>
<td>20</td>
<td>70</td>
<td>58.1±12.9</td>
<td>59</td>
<td>n.a.</td>
<td>27</td>
<td>31</td>
<td>41</td>
<td>87</td>
<td>100</td>
<td>29</td>
<td>n.a.</td>
<td>ESRD on HD</td>
<td>CD34* KDR+</td>
<td>CVE (4.4%) All death (4.2%)</td>
</tr>
<tr>
<td>Maruyama et al⁵</td>
<td>75</td>
<td>Japan</td>
<td>23</td>
<td>216</td>
<td>65±11</td>
<td>56.4</td>
<td>29.6</td>
<td>n.a.</td>
<td>n.a.</td>
<td>48.6</td>
<td>72.7</td>
<td>100</td>
<td>12.5</td>
<td>40.3</td>
<td>ESRD on HD</td>
<td>CD34*</td>
<td>CVE (10.3%) All death (3.1%)</td>
</tr>
<tr>
<td>Lorenzen et al⁵</td>
<td>75</td>
<td>Germany</td>
<td>36</td>
<td>265</td>
<td>66±15</td>
<td>55.4</td>
<td>9.8</td>
<td>84.5</td>
<td>34.3</td>
<td>81.8</td>
<td>100</td>
<td>56.6</td>
<td>29</td>
<td>n.a.</td>
<td>ESRD on HD</td>
<td>CFU</td>
<td>CVE (13.7%)</td>
</tr>
<tr>
<td>Lu et al²</td>
<td>75</td>
<td>Taiwan</td>
<td>50</td>
<td>154</td>
<td>69±15.3</td>
<td>46.7</td>
<td>16.2</td>
<td>n.a.</td>
<td>n.a.</td>
<td>55.8</td>
<td>68.8</td>
<td>100</td>
<td>n.a.</td>
<td>n.a.</td>
<td>ESRD on HD</td>
<td>CD34<em>CD133</em>KDR+ CVE death (6.3%) All death (12.2%)</td>
<td></td>
</tr>
<tr>
<td>Weimer et al²</td>
<td>75</td>
<td>Germany</td>
<td>12</td>
<td>507</td>
<td>66.6±10.8</td>
<td>67.1</td>
<td>22.9</td>
<td>39</td>
<td>79.3</td>
<td>29</td>
<td>85.2</td>
<td>17.9</td>
<td>55.2</td>
<td>55.4</td>
<td>CD34* KDR+</td>
<td>CVE (42.2%)</td>
<td></td>
</tr>
<tr>
<td>Alba et al³</td>
<td>62.5</td>
<td>Canada</td>
<td>7</td>
<td>156</td>
<td>55±11</td>
<td>77</td>
<td>n.a.</td>
<td>7</td>
<td>59</td>
<td>24</td>
<td>42</td>
<td>100</td>
<td>54</td>
<td>92</td>
<td>Chronic HF</td>
<td>CD34* KDR+</td>
<td>CVE (70.3%) All death (13.1%)</td>
</tr>
<tr>
<td>Fadini et al⁷</td>
<td>68.7</td>
<td>Italy</td>
<td>34</td>
<td>214</td>
<td>56.3±13</td>
<td>55.1</td>
<td>21</td>
<td>34.5</td>
<td>44.3</td>
<td>36.9</td>
<td>52.3</td>
<td>9.8</td>
<td>n.a.</td>
<td>n.a.</td>
<td>MS+healthy</td>
<td>CD34*</td>
<td>CVE (6.1%) All death (3.4%)</td>
</tr>
</tbody>
</table>

(Continued)
Similar results were obtained when the analysis excluded studies wherein the risk estimate had to be calculated, whereas limiting the analysis to higher quality studies yielded a risk ratio of RR 4.33 (4.01–4.69) for restenosis associated with low versus high cell count (Online Figure IIC). According to the fixed-effect model, the risk of restenosis associated with a low CPC/EPC cell count would be 2.97 (2.77–3.17).

Data on the risk for future revascularization were available only for 3 phenotypes: there was a nonsignificant trend association of reduced risk in patients with low CPCs/EPCs, but heterogeneity was high and statistically significant (Figure 4B). Excluding studies conducted in acute CVD patients or studies for which the risk estimate had to be calculated did not change the results using the random-effect model (Online Figure IID) although it yielded a significant risk ratio using the fixed-effect model (RR, 1.21 [1.02–1.43]).

Metaregression Analysis
A metaregression was conducted to detect whether any overall characteristic of study populations consistently modulated the risk estimate. As robustness of metaregression relies on the number of studies included, we only analyzed the risk ratio for the most commonly used progenitor cell phenotype (CD34+KDR+ EPCs) and the most common outcome (CVE). With the random-effect model, the log of risk ratio was significantly associated with the percentage of male patients (direct correlation, \( P < 0.0001 \)); Figure 5). No statistically significant outlier was detected in this metaregression.

Excluded Studies
Excluded studies reported data on the relation between CPC/EPC levels and functional outcome after acute myocardial infarction or stroke. Four studies were conducted on a total of \( n = 309 \) patients with acute myocardial infarction, overall showing that high CD34+/CD133+ CPCs predicted improvements in regional or global left ventricular function,37–39 and in coronary flow reserve.40 One study found that mobilization of EPCs during acute ischemia was significantly lower in patients who developed restenosis,41 whereas a small study in patients with stable angina showed that EPC levels directly correlated with the degree of restenosis.42 In 1 study conducted in patients with acute ischemic stroke, a low number of baseline EPCs predicted worse functional outcomes after 6 months.43

Biases
The number of studies available for which a given CPC/EPC phenotype was assessed in relation to a given outcome was limited. In funnel plots showing all phenotypes simultaneously (Online Figure III), there is a suggestion of missing studies on the bottom left-hand side of the plot. Because most of this area contains regions of high significance, publication bias is unlikely to be the underlying cause of asymmetry. Heterogeneity likely arose from selective outcome and analysis reporting, and poor quality of some studies.14

Discussion
This is the first systematic meta-analysis reporting pooled data on the association between levels of CPCs, cardiovascular outcomes,
and death. The overall analysis, obtained pooling risk ratios for all phenotypes, shows that a reduced baseline CPC/EPC level is associated with a \( \approx 2 \) fold increased risk of a combined CVE end point (a modified MACE) and all-cause mortality.

Among individual phenotypes, those most frequently associated with cardiovascular outcomes and death were CD34+ and CD34+CD133+. Such cell populations are mostly of hematopoietic origin, whereas EPCs, despite their vascular specification, were less consistently associated with the outcome. This discrepancy was noted previously and has different potential explanations. First, circulating CPCs better reflect the BM status than EPCs. Second, KDR+ EPCs are rarer than CPCs, show

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Forest plot of the risk for future cardiovascular events associated with a low vs a high circulating progenitor cell/endothelial progenitor cell count. Separated results are shown for each different phenotype of progenitor cells. Therefore, studies reporting risk estimates associated with the levels of different phenotypes are reported more than once. The group called overall reports subtotal risk estimates for each phenotype and a pooled risk ratio. Weights of each study, and individual risk ratios with 95% confidence interval (CI) are shown, along with tests for heterogeneity and overall effect. CFU indicates colony-forming cells; and IV, inverse variance.
larger variations, and their measure has higher variability. This is particularly true for enumeration of CD34+CD133+KDR+ EPCs, which requires a large number of event scoring by flow cytometry. Furthermore, anti-KDR antibodies are not clinical-grade and lot-by-lot variations may occur. Quantification of CD34+ cells using various modifications of the International Society for Hematotherapy and Graft Engineering (ISHAGE) protocol is routinely available in most hospital laboratories and already used in hematology clinical practice. Therefore, the CD34+ CPC phenotype is candidate to become a clinical-grade biomarker of cardiovascular risk.

Despite preclinical studies offer mechanistic interpretations for the link between low CPC and adverse outcomes, these remain largely speculative. As hematopoietic progenitor cells outperformed EPCs’ prognostic power and predicted all-cause mortality in addition to CVE, other, nonvascular, mechanisms may link stem/progenitor cell defects to adverse outcomes. For instance, circulating CPCs reflect the status of the BM, which is typically affected in syndromes characterized by premature death. Therefore, we speculate that reduced CPCs may generally reflect biological aging.

Some studies evaluating the prognostic capacity of CPCs/EPCs had to be excluded from the meta-analysis because not reporting risk measures. Nonetheless, their main message is that low progenitor cell levels are associated with poor functional outcomes after myocardial infarction or stroke, thereby lending support to the core finding of this meta-analysis.

Two studies in the literature show that, in addition to being associated with a significant risk of future CVD, the measure of circulating progenitors improves risk stratification beyond traditional assessment, as determined by the net reclassification improvement and the integrated discrimination improvement. This information further supports the clinical utility of measuring CPCs.

Limitations of this meta-analysis need to be acknowledged. Although the possibility to pool data from different cohorts and different phenotypes has been demonstrated, the meaning of pooling risk ratios from all phenotype is unclear. Quality of studies was low for some of the items identified by the REMARK guidelines. Studies were highly heterogeneous in terms of setting, patient population, underlying clinical condition, outcome definition, duration of follow-up, and progenitor cell phenotype. In the presence of large heterogeneity, the random-effect model is preferred, but analyzing eventual differences with the fixed-effect model may be important. Results of the random- and fixed-effect model were similar, suggesting a limited impact of heterogeneity on the overall message of the study. Furthermore, limiting the analysis to higher quality studies might further support the clinical utility of measuring CPCs.

Figure 3. Forest plot of cardiovascular (A) and all-cause (B) mortality risk associated with a low vs a high circulating progenitor cell/endothelial progenitor cell count. Separated results are shown for each different phenotype of progenitor cells. Weights of each study, and individual risk ratios with 95% confidence interval (CI) are shown, along with tests for heterogeneity and overall effect. IV indicates inverse variance.
studies yielded a significant risk of adverse outcomes associated with low progenitor cells. Nonetheless, differences among studies translated into a statistically significant heterogeneity of the risk estimate. This was most evident for restenosis and revascularization. The association between low CPCs/EPCs and reduced risk for future revascularization is counterintuitive. Methodologically, differences in the clinical setting (acute versus chronic CVD) and in progenitor cell phenotype may account for this discrepancy. Biologically, reduction of vascular-protective cells may prevent successful revascularization, by inducing a more severe and occlusive atherosclerosis, less amenable to surgical or endovascular intervention. Furthermore, in 7 of 21 studies, the risk ratio reported in the original article had to be re-elaborated or extrapolated from other statistics to be entered in the meta-analysis. Excluding such studies increased statistical significance and reduced heterogeneity. All meta-analyzed studies included patients at a high cardiovascular risk at baseline, mostly in secondary prevention, and 5 studies enrolled only patients with acute CVD, who have the highest short-term cardiovascular risk. As the CPC/EPC enumeration is usually

Figure 4. Forest plot of the risk of restenosis (A) and revascularization (B) associated with a low vs a high circulating progenitor cell/endothelial progenitor cell count. Separated results are shown for each different phenotype of progenitor cells. Weights of each study, and individual risk ratios with 95% confidence interval (CI) are shown, along with tests for heterogeneity and overall effect. CFU indicates colony-forming cells; and IV, inverse variance.

Figure 5. Metaregression analysis. The natural log of the risk ratio for cardiovascular events is plotted against various characteristics of each study. Size of the bubbles is proportional to the weight of each study according to the random-effect model. The regression lines and P values are shown. AMI indicates acute myocardial infarction.
performed on fresh samples, to yield a sufficient statistical power in the analysis of event-free survival with relatively small sample size and short follow-up, high-risk patients need to be enrolled. Because biomarkers perform better in high-risk than in low-risk populations, whether the levels of CPCs/EPCs provide prognostic information also in the general population remains unclear. Some observations suggest that the prognostic power of CPCs/EPCs is proportional to the baseline cardiovascular risk. First, the exclusion of studies that enrolled only patients with acute events lowered the risk ratio for future CVE associated with low progenitor cells, which however remained statistically significant. Second, the metagression showed that the risk ratio was higher in studies wherein patients were more often men, had a higher prevalence of baseline CVD, and were less frequently on statins. Metaregression should be considered often men, had a higher prevalence of baseline CVD, and were less frequently on statins. Metaregression should be considered

In conclusion, this meta-analysis shows that a reduction in the levels of circulating cells provided with vasculoregenerative capacity in preclinical models represents a risk factor for adverse cardiovascular outcomes and death. Future longitudinal studies on the prognostic capacity of CPC levels should be multicentric, use standardized assessment of CD34+ cells on both fresh and frozen samples, conducted on lower risk populations, and have longer follow-up. The final challenge will be an assessment of whether the measure of CPCs can guide clinical decisions.

Sources of Funding

This study was supported by institutional grants from the Department of Medicine of the University of Padova.

Disclosures

None.

References


What New Information Does This Article Contribute? What Is Known?

- Circulating progenitor cells (CPCs) are immature cells, mostly of hematopoietic origin, derived from the bone marrow.
- CPCs have been associated with several aspects of cardiovascular disease, from pathophysiology to diagnosis and therapy.
- Some studies have analyzed the association between baseline CPC levels and future cardiovascular events.

- A formal meta-analysis of longitudinal studies assessing the prognostic impact of CPCs.
- Low versus high CPC levels independently predict cardiovascular and all-cause mortality and future cardiovascular events.
- High heterogeneity among studies limits the generalizability of the findings, but CPCs could be a potential biomarker for assessing cardiovascular disease.
Levels of Circulating Progenitor Cells, Cardiovascular Outcomes and Death: A Meta-Analysis of Prospective Observational Studies
Mauro Rigato, Angelo Avogaro and Gian Paolo Fadini

Circ Res. 2016;118:1930-1939; originally published online April 12, 2016;
doi: 10.1161/CIRCRESAHA.116.308366

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/12/1930

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2016/04/12/CIRCRESAHA.116.308366.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/
Rigato et al  Metanalysis of CPCs’ prognostic power

SUPPLEMENTARY MATERIAL
Online Table I. Definitions of 16 items of study reporting quality, modified from REMARK guidelines

<table>
<thead>
<tr>
<th>REMARK Guidelines 2006</th>
<th>Label for item</th>
<th>How the item was operationalised</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Give rationale for sample size”</td>
<td>Rationale for sample size</td>
<td>Was there a statistical sample size or power calculation</td>
</tr>
<tr>
<td>“State any pre-specified hypotheses”</td>
<td>Pre-specified hypothesis or study protocol</td>
<td>Was there a bibliographic reference (e.g. to a protocol) stating that studying the relation of CPCs with cardiovascular events was part of the rationale for collecting the patient sample; or was there a pre-specified analysis plan?</td>
</tr>
<tr>
<td>Population</td>
<td>Healthcare setting</td>
<td>What was the healthcare setting from which patients were recruited?</td>
</tr>
<tr>
<td>Describe inclusion and exclusion criteria</td>
<td>Exclusion criteria</td>
<td>Were exclusion criteria reported?</td>
</tr>
<tr>
<td>“Describe number of patients included in each stage of the analysis and reasons for dropout”</td>
<td>Number of patients included in each stage of the analysis and reasons for dropout</td>
<td>Was there a description of numbers of patients at different stages (a. the total number of patients who were invited to participate in the study and who met eligibility criteria, b. The subset with complete follow up and the reasons for dropout.</td>
</tr>
<tr>
<td>Biomarker measurement</td>
<td>Manufacturer</td>
<td>Was the name of the company which makes the materials for CPCs stated?</td>
</tr>
<tr>
<td>“Specify assay method and provide (or reference) protocol”</td>
<td>Assay</td>
<td>Was the type of assay used to measure CPCs described?</td>
</tr>
<tr>
<td>“Describe methods of storage”</td>
<td>Sample handling</td>
<td>Were CPCs measured in a fresh sample or if the blood was stored, was the temperature stated?</td>
</tr>
<tr>
<td>Confounders</td>
<td>Conventional risk factors and other markers measured</td>
<td>Were the following conventional risk factors measured: age, sex, smoking status, total cholesterol, low-density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, body mass index, diabetes AND was at least another marker measured (e.g. fibrinogen, IL-6 or white cell count)</td>
</tr>
<tr>
<td>Outcomes</td>
<td>Primary outcome</td>
<td>Was a single disease outcome, or a single combination of outcomes, defined as the primary outcome for the analysis?</td>
</tr>
<tr>
<td>Validation</td>
<td></td>
<td>Were outcome events cross checked by independent sources? E.g. examining clinical records and national routine data</td>
</tr>
<tr>
<td>Analytic decisions</td>
<td>Masking</td>
<td>Was the ascertainment and classification of outcomes blinded to the CPCs value and other clinical information?</td>
</tr>
<tr>
<td>“Present univariate analyses of relation between marker and outcome”</td>
<td>Univariate estimate</td>
<td>Was the effect of CPCs on outcome presented either crude, or adjusted for age, or adjusted for age and sex?</td>
</tr>
<tr>
<td>“Provide estimated effects... in which the marker and standard prognostic variables are included, regardless of their statistical significance”</td>
<td>Adjusted for all conventional risk factors</td>
<td>Were the conventional risk factors (listed above) included as adjustments, regardless of their statistical significance?</td>
</tr>
<tr>
<td>“Report distributions”</td>
<td>Comparison group rationale</td>
<td>Was a reason given for choosing to analyse the data as continuous or categorical and if cutpoints were used was the method for their selection clear?</td>
</tr>
<tr>
<td>“Clarify how marker values were handled in the analysis”</td>
<td>Comparison group rationale</td>
<td>Was a reason given for choosing to analyse the data as continuous or categorical and if cutpoints were used was the method for their selection clear?</td>
</tr>
<tr>
<td>“How were missing data handled”</td>
<td>Missing values</td>
<td>a. Was the number of patients with missing values for CPCs or confounders stated? AND b. Was it stated how missing values were dealt with in the analysis?</td>
</tr>
</tbody>
</table>
Online Table II. Studies wherein standardized RR was not directly provided. The risks expressed per unit of CPC / EPC change was standardized using the attributable risk approach. *only for CVE.

<table>
<thead>
<tr>
<th>Study &amp; ref.</th>
<th>Rationale reported in studies</th>
<th>Transformation</th>
<th>Rationale for meta-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiang et al. 2014</td>
<td>rate of events per low vs high cells (tertiles)</td>
<td>relative risk computation</td>
<td>RR low vs high cells (tertiles)</td>
</tr>
<tr>
<td>Pelliccia et al. 2013</td>
<td>number of events per low vs high cells (tertiles)</td>
<td>relative risk computation</td>
<td>RR low vs high cells (tertiles)</td>
</tr>
<tr>
<td>Briguori et al. 2010*</td>
<td>RR low vs high cells (per 1 cell HPF)</td>
<td>standardization with attributable risk approach</td>
<td>RR low vs high cells (per 1 delta SD)</td>
</tr>
<tr>
<td>Bonello et al. 2012</td>
<td>RR low vs high cells (per 1 delta%)</td>
<td>standardization with attributable risk approach</td>
<td>RR low vs high cells (per 1 delta SD)</td>
</tr>
<tr>
<td>Haine et al. 2014</td>
<td>RR low vs high cells (per 1 log)</td>
<td>standardization with attributable risk approach</td>
<td>RR low vs high cells (per 1 SD)</td>
</tr>
<tr>
<td>Lorenzen et al. 2010</td>
<td>RR high vs low cells (per 1 cell/HPF)</td>
<td>standardization with attributable risk approach</td>
<td>RR low vs high cells (per 1 SD)</td>
</tr>
<tr>
<td>Alba et al. 2013</td>
<td>RR high vs low cells (per 10 units)</td>
<td>standardization with attributable risk approach</td>
<td>RR low vs high cells (per 1 SD)</td>
</tr>
<tr>
<td>Yu et al. 2013</td>
<td>RR high vs low cells (per 1 cell/ul)</td>
<td>standardization with attributable risk approach</td>
<td>RR low vs high cells (per 1 SD)</td>
</tr>
</tbody>
</table>
**Online Table III.** Summary of characteristics of excluded studies. AMI: Acute Myocardial Infarction; LVEF: Left Ventricular Ejection Fraction; CFR: Coronary Flow Reserve; WMSI: Wall Motion Score Index.

<table>
<thead>
<tr>
<th>Study &amp; ref</th>
<th>Year</th>
<th>Sample size</th>
<th>Population</th>
<th>Phenotype</th>
<th>Outcomes</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leone et al. 1</td>
<td>2005</td>
<td>28</td>
<td>AMI</td>
<td>CD34⁺</td>
<td>LVEF changes from baseline</td>
<td>RR not provided</td>
</tr>
<tr>
<td>Jeong et al. 2</td>
<td>2012</td>
<td>110</td>
<td>AMI</td>
<td>CD34⁺</td>
<td>CFR changes from baseline</td>
<td>RR not provided</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD133⁺</td>
<td>CFR changes from baseline</td>
<td>RR not provided</td>
</tr>
<tr>
<td>Wyderka et al. 3</td>
<td>2012</td>
<td>50</td>
<td>AMI</td>
<td>CD34⁺</td>
<td>LVEF changes from baseline</td>
<td>RR not provided</td>
</tr>
<tr>
<td>Wojakowski et al. 4</td>
<td>2013</td>
<td>60</td>
<td>AMI</td>
<td>CD34⁺CD133⁺KDR⁺</td>
<td>Restenosis</td>
<td>RR not provided</td>
</tr>
<tr>
<td>Grąbczewska et al. 5</td>
<td>2013</td>
<td>61</td>
<td>AMI</td>
<td>CD34⁺</td>
<td>WMSI changes from baseline</td>
<td>RR not provided</td>
</tr>
<tr>
<td>Tsai et al. 6</td>
<td>2013</td>
<td>65</td>
<td>Acute Stroke</td>
<td>CD34⁺KDR⁺</td>
<td>Stroke outcome</td>
<td>RR not provided</td>
</tr>
<tr>
<td>De Maria et al. 7</td>
<td>2014</td>
<td>20</td>
<td>Stable Angina</td>
<td>CD34⁺KDR⁺</td>
<td>Restenosis</td>
<td>RR not provided</td>
</tr>
</tbody>
</table>
**Online Table IV.** Summary of characteristics of all the patients (n=4,155) included in the meta-analysis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up months (weight mean±SD)</td>
<td>22.4±9.7</td>
</tr>
<tr>
<td>Age, years (weight mean±SD)</td>
<td>63.8±11.6</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>63.7</td>
</tr>
<tr>
<td>Smoke (%)</td>
<td>39.9</td>
</tr>
<tr>
<td>Previous CVD (%)</td>
<td>34.5</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>62.8</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>31.5</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>69.7</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>58.4</td>
</tr>
<tr>
<td>Ace-i/ARB (%)</td>
<td>50.5</td>
</tr>
</tbody>
</table>
SUPPLEMENTARY FIGURES

Online Figure I. Quality of studies included in the meta-analysis. The percentage of studies satisfying each of the 16 items modified from the REMARK guidelines is reported. Studies excluded from meta-analysis (n=7) are not shown.
Online Figure II. Subanalyses. For cardiovascular events (a), all-cause mortality (b), restenosis (c), and revascularization (d), up to 5 subanalyses are reported. Subanalysis 1 was performed excluding studies conducted only in patients with acute CVD. Subanalysis 2 was performed excluding studies for which the risk estimate had to be calculated (see Online table II). Subanalysis 3 was performed only including studies conducted only in patients with acute CVD. Subanalysis 4 was performed only with higher quality studies (>10 REMARK items). Subanalysis 5 was performed only with studies having a homogeneous definition of the composite cardiovascular endpoint (>50% hard events).
Online Figure III. Funnel plots. Funnel plots have been constructed for all phenotypes versus cardiovascular events and all-cause mortality.
REFERENCES


5. Grabczewska Z, Debski R, Goralczyk K, Swiatkiewicz I, Kubica J. Does mobilisation of cd34+ stem cells along with vegf, angiogenin, il-6, il-8, and hscrp levels allow predicting the direction of left ventricular ejection fraction and wall motion score index changes in patients with myocardial infarction? *Kardiol Pol*. 2013;71:464-471
