Background: This study explored the clinical significance of CD34+/133+ circulating progenitor cell (CPC) counts in patients with stable angina pectoris (AP) who underwent percutaneous coronary intervention (PCI).

Methods and Results: Subjects comprised 52 patients with stable AP requiring PCI and 50 control patients without AP. In the AP group, blood samples were taken before and 20 min and 24 h after PCI to measure CPC counts by fluorescence-activated cell sorter analysis. The baseline number of CPCs was smaller in the AP group than in controls. In the AP group, body mass index (BMI) correlated positively with the baseline number of CPCs and was an independent predictor of CPC count in multivariate regression analysis. Other conventional risk factors, daily exercise activity and statin administration showed no association with CPC count. CPC counts remained unchanged within 24 h after PCI.

Conclusions: CPC counts in patients with AP are influenced by BMI, but not by other coronary risk factors. CPC counts remain unchanged within 24 h after PCI. (Circ J 2010; 74: 1929–1935)

Key Words: Coronary artery disease; Endothelium; Exercise; Percutaneous coronary intervention; Prognosis

Circulating CD34+/133+ Progenitor Cells in Patients With Stable Angina Pectoris Undergoing Percutaneous Coronary Intervention

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B one marrow-derived endothelial progenitor cells (EPCs) are found in peripheral blood, and can be incorporated into injured and new vasculature.\textsuperscript{1,2} The numbers and function of EPCs, which are assumed to reflect endothelial function and endogenous vascular repair capacity, have been reported to predict the occurrence of cardiovascular disease.\textsuperscript{3–8} Although no uniform definition regarding EPCs has been accepted, quantification by flow cytometry is a simple and pragmatic approach in the clinical setting. Using this method, we have previously found that moderate daily exercise for >4 h/week increases the number of circulating CD34+/133+ progenitor cells (CPCs) and exercise capacity 3 months after the onset of AMI,\textsuperscript{9} and CPC counts appear to correlate positively to exercise capacity, but not to reduction of restenosis with bare metal stent (BMS) implantation. Banerjee et al recently showed that CD34/CD31-positive EPC colony-forming units in the peripheral circulation are increased at 12 h after percutaneous coronary intervention (PCI) in patients with stable angina pectoris (AP), but not in patients with acute coronary syndrome.\textsuperscript{10} Although EPCs are important for repairing denuded endothelium in the vessels injured by PCI, the clinical significance of CPC counts in stable AP patients undergoing PCI remains unclear.\textsuperscript{9,10}

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The present study examined the clinical significance of the CPC count quantified by flow cytometry as a biomarker in patients with stable AP undergoing PCI. The specific aims were to explore factors contributing to the number of CPCs and acute mobilization of CPCs after PCI.

Study Population

The patient group in this multicenter, prospective, observational study comprised 52 consecutive patients with stable AP who were admitted to hospital between March and July 2006 to undergo elective catheter coronary revascularization with balloon (POBA, n=5) and/or BMS (n=9) and/or sirolimus-eluting stent (SES, n=38). Another 50 age-matched
patients without coronary artery disease documented by coronary arteriography and/or exercise stress scintigraphy were enrolled as controls. All patients in the control group were hospitalized between March 2006 and July 2009 for evaluation of ischemic heart disease or treatment of arrhythmia, diabetes mellitus or dyslipidemia. Exclusion criteria were acute coronary syndrome, left ventricular ejection fraction <40% and age >80 years. All patients gave written informed consent to the study protocol, which was approved by the internal ethical committees.

Blood Sampling and Assay
Fasting blood samples were taken from an antecubital vein at rest before PCI or electrophysiological study, and 20 min and 24 h after PCI. Trained technicians measured the numbers of CD45^low^/34^+^/133^+^ cell as CPCs, using a fluorescence-activated cell sorting (FACS) system (FACSCalibur; BD Biosciences, CA, USA) with anti-human CD34^+^, CD45^+^ and CD133^+^ antibodies (BD Biosciences) as described previously. Because baseline CPC counts in peripheral blood did not show a normal distribution (Figure 1a), data are expressed as log_{10} CPC count. High-sensitivity C-reactive protein (hsCRP) was measured using a monoclonal antibody employing latex (Nanopia CRP; Daiichi Pure Chemical, Tokyo, Japan). Serum levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol and plasma glucose were measured using enzymatic methods. Hemoglobin (Hb) A_1c levels were assessed by high-performance liquid chromatography.

PCI Procedure
Most PCI procedures were performed as follows. First, the lesion was pre-dilated with a balloon at nominal pressure after wire passage. Second, a BMS or SES was deployed at almost the rated burst pressure after evaluation by intravascular ultrasonography. If necessary, post-dilation at high pressure was added.

Definitions of Coronary Risk Factors
Coronary risk factors were identified from the medical history or the following hospital data: hyperlipidemia, LDL-C >140 mg/dl; hypertension, systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg; diabetes mellitus, HbA_1c >6.5%; and obesity, body mass index (BMI) ≥25.0 in accordance with the definition of the Japan Society for the Study of Obesity.

Semi-Quantification of Weekly Exercise
The weekly exercise activity of each patient was quantified by inquiry. One exercise day was defined as walking or jogging exercise for >20 min/day. No significant differences in log_{10} CD34^+^/133^+^ cell counts were seen among the 3 groups (0–1 day/week, 2–3 days/week and 4–7 days/week).

Statistical Analysis
Continuous variables that did not show normal distribution were statistically analyzed after logarithmic conversion and were described as mean± standard deviation (SD), and were compared using 2-way analysis of variance or Student’s t-test, as appropriate. Categorical variables were described as frequencies and were compared using χ^2 analysis. Correlations were assessed using Fisher’s coefficient (r). Values of
Table 1. Clinical Characteristics of the Study Patients

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Angina</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>50 (36/14)</td>
<td>52 (41/11)</td>
<td>0.566</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68±10</td>
<td>66±12</td>
<td>0.153</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24±4</td>
<td>25±4</td>
<td>0.491</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>16 (32)</td>
<td>22 (42)</td>
<td>0.335</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>21 (42)</td>
<td>29 (56)</td>
<td>0.233</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>25 (50)</td>
<td>31 (60)</td>
<td>0.437</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>26 (52)</td>
<td>29 (56)</td>
<td>0.855</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>24 (48)</td>
<td>20 (38)</td>
<td>0.240</td>
</tr>
<tr>
<td>Administration of statins, n (%)</td>
<td>12 (24)</td>
<td>20 (44)</td>
<td>0.174</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>202±29</td>
<td>193±29</td>
<td>0.116</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>142±76</td>
<td>143±92</td>
<td>0.994</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>117±26</td>
<td>111±26</td>
<td>0.217</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>52±12</td>
<td>48±15</td>
<td>0.112</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.3±1.2</td>
<td>6.4±1.7</td>
<td>0.882</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>0.28±0.35</td>
<td>0.33±0.56</td>
<td>0.822</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.8±0.2</td>
<td>1.2±1.4</td>
<td>0.158</td>
</tr>
<tr>
<td>No. of coronary risk factors</td>
<td>2.5±1.3</td>
<td>2.3±1.5</td>
<td>0.458</td>
</tr>
<tr>
<td>No. of CD34+/CD133⁺ cells (/100 μl)</td>
<td>67 (37–102)</td>
<td>52 (22–99)</td>
<td></td>
</tr>
<tr>
<td>Log₁₀CD34+/CD133⁺ cell number</td>
<td>1.82±0.20</td>
<td>1.71±0.24</td>
<td>0.0096</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD or median and 10th to 90th percentile. Blood samples were taken in the morning in a fasting state.

BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycohemoglobin A1c; hsCRP, high-sensitivity C-reactive protein.

Figure 2. Effects of sex, diabetes mellitus, hypertension, hyperlipidemia, current smoking and administration of statins on differences in CD34+/133⁺ cell numbers in peripheral blood. For obesity, we adopted the definition of the Japan Society for the Study of Obesity (body mass index (BMI) ≥25.0) rather than that of the International Association for the Study of Obesity (BMI ≥30.0), as all patients were Japanese. No factors displayed any significant effect on CD34+/133⁺ cell numbers.
Figure 3. Correlation of CD34+/133+ cell numbers with (a) age and (b) body mass index (BMI). The number of CPCs correlated negatively with age (r=-0.102, P=0.0083) and positively with BMI (r=4.51, P=0.0003).

Table 2. Correlation of Each Coronary Risk Factor With Baseline log_{10} CD34+/133+ Cell Number

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P value</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.305</td>
<td>0.031</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>-0.001</td>
<td>0.571</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.0004</td>
<td>0.783</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-0.024</td>
<td>0.243</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>-0.009</td>
<td>0.882</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>0.402</td>
<td>0.004</td>
</tr>
<tr>
<td>No. of coronary risk factors</td>
<td>0.022</td>
<td>0.486</td>
</tr>
</tbody>
</table>

R, regression coefficient. Other abbreviations see in Table 1.

Figure 4. Correlation between number of coronary risk factors and (a) body mass index (BMI) and (b) log_{10} CD34+/133+ cell numbers. BMI showed a positive correlation with the number of coronary risk factors (r=0.106, P=0.011). No significant correlation was identified between the number of coronary risk factors and log_{10} CD34+/133+ cell numbers.
P<0.05 were considered indicative of statistical significance. Statistical analysis was performed using StatView 5.0 software (SAS Institute, Cary, NC, USA).

**Results**

Baseline clinical characteristics of all patients are shown in Table 1. The baseline log_{10} CPC count was significantly lower in the AP group than in the controls (Table 1, P=0.0096). During the study period, none of the subjects in the AP group showed a significant elevation of serum creatine kinase more than twice the upper limit of normal. For the AP patients, no significant differences in baseline log_{10} CPC count were seen among the 3 exercise groups (Figure 1b). Although obese patients showed higher log_{10} CPC counts than non-obese patients, no significant differences in log_{10} CPC counts were identified between the presence and absence of conventional coronary risk factors such as diabetes mellitus, hypertension, dyslipidemia, current smoking or administration of statins (Figure 2). Log_{10} CPC counts tended to be larger in men than in women (P=0.10; Figure 3).

In the univariate regression analysis, log_{10} CPC count before PCI correlated with age (Figure 3a; P=0.030) and BMI (Figure 3b; P=0.0038), but not with lipid profile or HbA1c (Table 2). In the multivariate regression analysis, only BMI was an independent attributable factor on log_{10} CPC counts (Table 2, P=0.035). No significant correlation was identified between the number of coronary risk factors and log_{10} CPC count (Figure 4).

The time course of log_{10} CPC count was similar among the 3 PCI groups treated by BMS, SES and POBA. Log_{10} CPC counts showed no significant changes at 20 min and 24 h after PCI as compared with baseline data (Figure 5).

**Discussion**

The present data show that: (1) baseline CPC count was decreased in AP compared with controls, although CPC count did not predict the long-term prognosis of stable AP patients undergoing PCI; (2) BMI was an independent predictor of CPC count in AP, although other coronary risk factors were not; and (3) no significant changes in CPC counts occurred within 24 h after PCI.

**Feasibility of CPC Count as a Biomarker**

EPC quantification by flow cytometry is a simple and pragmatic approach compared with cell cultures. However, true EPCs (late outgrowth endothelial progenitors) as defined by CD34+/CD133+/VEGFR2+ are exceedingly rare in peripheral blood, with an estimated frequency in healthy volunteers of 1 EPC per 20 ml of blood according to our pilot study and previous studies by other investigators.6,11,12 In our pilot study, the number of CD34+/CD133+/VEGFR-2+ cells by FACS was 0/100 μl in 52 of 80 patients with ischemic heart disease, which means that we need to sample at least 100 ml of blood for quantification of true EPCs. Instead of the CD34+/CD133+/VEGFR2+ cell count, the CD34+/CD133+ cell count is thought to offer a more practical alternative as a clinical biomarker.9,13 The decreased CPC count in patients with AP compared with controls in our study may reflect the impaired capacity of vascular repair in AP patients.5,6,14

**Coronary Risk Factors and CPC Count in Patients With Stable AP**

The cumulative number of coronary risk factors, which is known to be predictive of future cardiovascular events, showed no significant association with baseline CPC count in patients with stable AP. Previous reports on the relationship between the cumulative number of risk factors and CPC or EPC counts have been controversial.5,9,10,14,15 Hill et al reported that the EPC count defined as colony-forming units on culture was inversely correlated with combined Framingham risk factor score in men without a previous history of heart disease at approximately 50 years old.3 In contrast, Xiao et al showed that EPC count, defined as double-positive-stained cells for Dil-Ac-LDL and lectin on culture, increased slightly with Framingham risk score in a population-based study.15 These divergent results may be attributable to the lack of a uniform definition for EPCs and differences in the subject populations.

Among the coronary risk factors, age showed an inverse relationship with baseline CPC count in our univariate analysis, in line with previous studies.16,19 CPC or EPC counts may decline with advancing age simply because of exhaustion of...
stem cells and coexisting atherosclerosis. The level of daily exercise did not affect baseline CPC counts in patients with stable AP, although intensive exercise training has been reported to increase the number of circulating EPCs.\textsuperscript{9,20–22} Even in the high exercise group in the present study, the degree of exercise was much less (>80 min/week) than the highest exercise group in our previous study (>240 min/week) of patients with myocardial infarction.\textsuperscript{3} Statins have a contradictory effect on the number of circulating EPCs: increasing the number and functional activity of EPCs at almost 4 weeks because of mobilization from bone marrow,\textsuperscript{23,24} but decreasing these values at >8 weeks after exhausting mobilization.\textsuperscript{25} In fact, statin administration prior to PCI did not affect CPC count in the present study. Our data showing a roughly positive correlation between BMI and CPC count is not consistent with some previous reports.\textsuperscript{3,25,27} A possible explanation is that only 2 severely obese patients (BMI ≥30) were included in the present study. A recent cross-sectional study demonstrated that CPC count was decreased in a severely obese group (BMI ≥30) compared with a normal weight group (BMI ≤25),\textsuperscript{28} although CPC count did not differ at all between the normal weight group (BMI ≤25) and moderately overweight group (BMI 25–29.9).\textsuperscript{29} EPCs have leptin receptors and leptin from adipose tissue has been reported to induce EPC migration.\textsuperscript{29,30} Unfortunately, we did not measure plasma levels of leptin in the present study.

**Serial Changes in CPC Count After PCI**

CPC counts were unchanged for 24 h after PCI, which coincides with the findings of a previous report concerning progenitor cells defined as CD34\textsuperscript{+} CPCs in home on denuded arteries after focal coronary endothelial injury as a result of PCI. Banerjee et al showed that CD34/CD31-positive CPC colony-forming units in the peripheral circulation are increased at 12 h after PCI in patients with stable AP (n=20), without any corresponding rise in vascular endothelial growth factor, which mobilizes EPCs from the bone marrow.\textsuperscript{31} Interpretation of the circulating CPC count is thus more complex than previously assumed in patients with AP. Further studies are warranted to elucidate whether CPC count offers a useful biomarker for secondary prevention.

**Study Limitations**

First, we measured CPC count using FACS only. Second, the SD for the measurements was somewhat large, because of the relatively small sample size. Third, to explore acute mobilization of CPCs after PCI, CPC numbers were measured at only 3 time points (just before PCI, and 20 min and 24 h after PCI).

**Conclusion**

CPC count in AP, which was decreased in comparison with controls, was influenced by age and BMI, but not by other risk factors. No significant changes in CPC counts were noted within 24 h after PCI.

**Acknowledgments**

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**References**

Circulating Progenitor Cells in AP


