ORIGINAL ARTICLE

Circulating Endothelial Progenitor Cells and Cardiovascular Outcomes

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ABSTRACT

BACKGROUND

Endothelial progenitor cells derived from bone marrow are believed to support the integrity of the vascular endothelium. The number and function of endothelial progenitor cells correlate inversely with cardiovascular risk factors, but the prognostic value associated with circulating endothelial progenitor cells has not been defined.

METHODS

The number of endothelial progenitor cells positive for CD34 and kinase insert domain receptor (KDR) was determined with the use of flow cytometry in 519 patients with coronary artery disease as confirmed on angiography. After 12 months, we evaluated the association between baseline levels of endothelial progenitor cells and death from cardiovascular causes, the occurrence of a first major cardiovascular event (myocardial infarction, hospitalization, revascularization, or death from cardiovascular causes), revascularization, hospitalization, and death from all causes.

RESULTS

A total of 43 participants died, 23 from cardiovascular causes. A first major cardiovascular event occurred in 214 patients. The cumulative event-free survival rate increased stepwise across three increasing baseline levels of endothelial progenitor cells in an analysis of death from cardiovascular causes, a first major cardiovascular event, revascularization, and hospitalization. After adjustment for age, sex, vascular risk factors, and other relevant variables, increased levels of endothelial progenitor cells were associated with a reduced risk of death from cardiovascular causes (hazard ratio, 0.31; 95 percent confidence interval, 0.16 to 0.63; P=0.001), a first major cardiovascular event (hazard ratio, 0.74; 95 percent confidence interval, 0.62 to 0.89; P=0.002), revascularization (hazard ratio, 0.77; 95 percent confidence interval, 0.62 to 0.95; P=0.02), and hospitalization (hazard ratio, 0.76; 95 percent confidence interval, 0.63 to 0.94; P=0.01). Endothelial progenitor-cell levels were not predictive of myocardial infarction or of death from all causes.

CONCLUSIONS

The level of circulating CD34+KDR+ endothelial progenitor cells predicts the occurrence of cardiovascular events and death from cardiovascular causes and may help to identify patients at increased cardiovascular risk.

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Coronary artery disease results from a chronic inflammatory disease of the vascular wall and leads to vessel occlusion and organ damage.[1] Despite intense efforts to determine the pathogenesis of atherosclerosis, this process remains poorly understood. Reports suggest that risk factors and a genetic predisposition together induce inflammatory processes that lead to cell damage and impair regeneration within the vessel wall.[2,3] Since resident endothelial cells infrequently proliferate,[4] it has been postulated that there are other sources of vascular replenishment in response to continuous damage.[5] Endothelial progenitor cells derived from bone marrow circulate in the peripheral blood and have been implicated in neoangiogenesis after tissue ischemia has occurred.[6-9] Endothelial progenitor cells are capable of proliferating and differentiating into endothelial cells and are therefore ideal candidates for vascular regeneration.[10,11] Experiments in animals show that the systemic application or mobilization of stem cells and progenitor cells beneficially influences the repair of endothelial cells after injury and the progression of atherosclerosis.[12-18] In humans, the role of endothelial progenitor cells is less clear. Intracoronary injection of endothelial progenitor cells may improve left ventricular function after acute myocardial infarction.[19-21] In addition, the accumulation of cardiovascular risk factors or an increased overall risk is associated with dysfunction and decreased numbers of endothelial progenitor cells.[22,23]

Although these data suggest that there is a close interplay between endothelial progenitor cells and cardiovascular risk factors, the exact role of these cells in the pathogenesis of coronary artery disease remains to be determined. It is unknown whether the number of endothelial progenitor cells relates to outcomes in patients with coronary artery disease. In order to test this hypothesis, we assessed the number of endothelial progenitor cells in patients with coronary artery disease and prospectively analyzed cardiovascular outcomes during a follow-up period of 12 months.

METHODS

STUDY POPULATION

Between March 2003 and January 2004, 587 patients who consecutively underwent coronary angiography were screened for inclusion in the Endothelial Progenitor Cells in Coronary Artery Disease study. Forty-nine patients without signs of coronary artery disease on angiography and 19 patients with malignant, inflammatory diseases or severe acute ischemia other than myocardial ischemia were excluded from the study. Informed consent was obtained from all patients, and the study protocol was approved by the ethics committee of the University of Saarland. The investigators initiated the study, had full access to and analyzed the data, and wrote the manuscript. All authors vouch for the data and analysis.

ANGIOGRAPHY

Cardiac catheterization was performed according to the guidelines for coronary angiography of the American College of Cardiology and the American Heart Association.[24] Biplane ventriculography was performed in standard projections. The ejection fraction was calculated by dividing the end-diastolic and end-systolic left ventricular areas with the use of an automated computer system (Digital Cardiac Imaging software, Philips). The extent of coronary artery disease was scored, by at least two independent interventional cardiologists, as 0 (stenosis <50 percent), 1 (stenosis of any main coronary artery ≥50 percent), 2 (stenosis of two main coronary arteries ≥50 percent), and 3 (stenosis of three main coronary arteries ≥50 percent).

PREVIOUS EVENTS, FOLLOW-UP, AND CAUSES OF DEATH

The classification of previous events and follow-up data was made on the basis of medical records and personal interviews. Causes of death were determined by examination of hospital records, autopsy reports, and medical files of the patients' general practitioners. Deaths due to cardiovascular causes included sudden deaths and deaths from acute myocardial infarction, coronary artery disease, or congestive heart failure.

PREPARATION OF BLOOD SAMPLES

Arterial blood was drawn from the femoral artery and buffered with 20 ml of sodium citrate before cardiac catheterization. Mononuclear cells were isolated with the use of a Ficoll density gradient (Biocoll, Biochrom) according to standard protocols. Additional blood samples were obtained for routine analyses.

FLOW CYTOMETRY

For fluorescence-activated cell-sorting analysis, mononuclear cells were resuspended in 100 μl of a fluorescence-activated cell-sorting buffer contain-

ing phosphate-buffered saline, 0.1 percent bovine albumin, and aprotinin (20 μl per milliliter). Immunofluorescent cell staining was performed with the use of the fluorescent conjugated antibody CD34–fluorescein isothiocyanate (FITC) (10 μl; Becton Dickinson), KDR (kinase insert domain receptor), and CD133–phycoerythrin (PE) (10 μl; Miltenyi). For the identification of KDR+ cells, indirect immunolabeling was performed with the use of a biotinylated goat mononuclear antibody against the extracellular domain of human KDR (R&D Systems). IgG2a–FITC–PE antibody (Becton Dickinson) served as a negative control. For staining of KDR, extensive blocking was required with the use of human immunoglobulin (polyglobulin, 10 percent; Bayer) and goat serum (Sigma-Aldrich). Cell fluorescence was measured immediately after staining, and data were analyzed with the use of CellQuest software (FACSCalibur, Becton Dickinson). Units of all measured components are absolute cell counts obtained after the measurement of 10,000 events in the lymphocyte gate. To assess the reproducibility of the measurements, two separate blood samples were obtained, on days 0 and 7, from 10 subjects. The intraclass correlation between the two probes was 0.94. Probes were measured at the same time of day, with identical instrument settings, by two investigators. For each patient, a corresponding negative control with IgG2a–FITC–PE antibody was obtained.

COLONY-FORMING UNITS OF ENDOTHELIAL CELLS

In an endothelial basal medium (CellSystems) with supplements, 1×10^7 mononuclear cells were seeded on human fibronectin–coated plates (Sigma-Aldrich). After 48 hours, 1×10^6 nonadherent cells were transferred into new fibronectin-coated wells to avoid contamination with mature endothelial cells and nonprogenitor cells.[22] After seven days in vitro, endothelial colony-forming units in at least three wells were counted by two independent investigators. Colony-forming units of endothelial cells are expressed as absolute numbers of colonies per well.

STATISTICAL ANALYSIS

The association between baseline levels of endothelial progenitor cells and the following prespecified end points was evaluated after 12 months: death from cardiovascular causes, the occurrence of a first major cardiovascular event (acute myocardial infarction, hospitalization due to cardiovascular events, revascularization, or death from cardiovascular causes), the need for revascularization, hospitalization due to cardiovascular events, and death from any cause. Levels of endothelial progenitor cells were analyzed as categorical variables after log transformation (on a base 10 scale) to normalize distribution. In categorical analyses, we used prespecified thresholds corresponding to patients' endothelial progenitor-cell counts (low, medium, and high) at the time of enrollment. Continuous variables were tested for normal distribution with the use of the Kolmogorov–Smirnov test. Means between two categories were compared with the use of a two-tailed, unpaired Student's t-test. The one-way analysis-of-variance test was used for comparisons of categorical variables. For post hoc analysis, the Bonferroni correction was applied. A multivariate proportional-hazards regression analysis was performed to determine the association between endothelial progenitor-cell counts and each outcome. Analyses were adjusted for age; sex; smoking status; the presence of hypertension, diabetes, or hyperlipidemia; left ventricular ejection fraction; percutanous coronary intervention; a diagnosis of an acute coronary syndrome at the time of enrollment; the severity of coronary artery disease; and treatment with angiotensin-converting–enzyme (ACE) inhibitors, beta-blockers, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), and platelet inhibitors. The hazard ratio represents the predicted change in the hazard for a unit increase in the predictor (e.g., an increase from low to medium or from medium to high in the number of endothelial progenitor cells). Survival was determined with the use of the Kaplan–Meier method and the Cox regression analysis. The log-rank test was used to determine statistical differences in terms of survival. Statistical significance was assumed when a null hypothesis could be rejected at $P<0.05$. Statistical analysis was performed with the use of SPSS software, version 11.5, for Windows.

All data analyses and event classifications were performed by investigators blinded to the endothelial progenitor-cell status of the patients.

RESULTS

BASELINE CHARACTERISTICS

A total of 519 patients with coronary artery disease as diagnosed on angiography were enrolled. Of these, 12 patients (2.3 percent) were lost to follow-up. The mean (±SD) age of the remaining 507

patients was 66.6±10.8 years (range, 30 to 87). Detailed characteristics of the patients are listed in Table 1.

ENDOTHELIAL PROGENITOR-CELL COUNTS AND BASELINE CLINICAL VARIABLES

The number of endothelial progenitor cells ranged from 12 to 1039 CD34+KDR+ cells, with a mean of 86.3±71.9. After logarithmic transformation (base 10), endothelial progenitor-cell counts were categorized into three groups according to the cell count at the time of enrollment (Table 1). Group 1 represents patients with log numbers of endothelial progenitor cells of 1.71 or less, group 2 patients with log numbers between 1.72 and 1.96, and group 3 patients with log numbers between 1.97 and 3.02.

In univariate analyses, smoking, diuretic therapy, and statin therapy were associated with high baseline levels of CD34+KDR+ endothelial progenitor cells (P=0.02, P=0.007, and P=0.05, respectively), whereas low levels were associated with a high left ventricular ejection fraction and treatment with angiotensin-receptor blockers (P=0.008 and P=0.03, respectively) (Table 1). In addition to CD34+KDR+ endothelial progenitor cells, we measured CD133+ cells, which resemble a subfraction of immature endothelial progenitor cells. Therapy with statins and ACE inhibitors was associated with high baseline levels of CD133+ cells (P=0.01 and P=0.03, respectively), whereas low levels were associated with increased low-density lipoprotein (LDL) cholesterol levels, advanced age, and high systolic blood pressure (P=0.01, P<0.001, and P=0.008, respectively).

In order to determine the functional capacity of circulating endothelial progenitor cells, we measured the number of colony-forming units of endothelial cells in a subgroup of 203 patients. Therapy with statins and ACE inhibitors was associated with increased numbers of colony-forming units of endothelial cells (P=0.001 and P=0.03, respectively), whereas reduced numbers of colony-forming units of endothelial cells were associated with increased LDL cholesterol levels, advanced age, diabetes, smoking, and a family history of premature coronary artery disease (P=0.01, P=0.002, P=0.01, P=0.002, and P=0.004, respectively).

INCIDENCE OF DEATH AND CARDIOVASCULAR EVENTS

Table 2 shows the incidence of outcomes during the 12 months of follow-up. A total of 43 patients (8.5 percent) died, 23 from cardiovascular causes (4.5 percent); other causes included sepsis (9 patients), chronic renal insufficiency (3), pneumonia (4), and cerebral bleeding (3). In one patient, the cause of death remained unclassified. Thirty-four patients (6.7 percent) had acute myocardial infarction, 163 (32.1 percent) required revascularization, and 186 (36.7 percent) were admitted to a hospital owing to cardiovascular events.

In univariate analyses, the incidence of death from cardiovascular causes was significantly influenced by advanced age (hazard ratio, 1.07; 95 percent confidence interval, 1.01 to 1.12; P=0.01), low left ventricular ejection fraction (hazard ratio, 0.96; 95 percent confidence interval, 0.94 to 0.99; P=0.004), concomitant treatment with beta-blockers (hazard ratio, 3.38; 95 percent confidence interval, 1.01 to 11.43; P=0.05), and a low level of circulating endothelial progenitor cells (hazard ratio, 0.45; 95 percent confidence interval, 0.25 to 0.81; P=0.007).

A multivariate regression analysis identified advanced age, low left ventricular ejection fraction, and a low level of circulating endothelial progenitor cells as the only independent predictors of death from cardiovascular causes. The occurrence of a first major cardiovascular event was significantly influenced by a greater severity of coronary artery disease (hazard ratio, 1.52; 95 percent confidence interval, 1.31 to 1.76; P<0.001), coronary intervention (hazard ratio, 1.57; 95 percent confidence interval, 1.20 to 2.06; P=0.001), a diagnosis of an acute coronary syndrome (hazard ratio, 1.51; 95 percent confidence interval, 1.13 to 2.02; P=0.006) or subacute myocardial infarction (hazard ratio, 1.57; 95 percent confidence interval, 1.03 to 2.40; P=0.04) at the time of enrollment, and a low level of circulating endothelial progenitor cells (hazard ratio, 0.72; 95 percent confidence interval, 0.61 to 0.86; P<0.001). In a multivariate analysis, the degree of coronary artery disease and the level of circulating endothelial progenitor cells were mutually independent predictors of the occurrence of a first major cardiovascular event.

ENDOTHELIAL PROGENITOR-CELL LEVELS AND CLINICAL OUTCOMES

Cumulative event-free survival increased in a stepwise fashion across increasing levels of baseline endothelial progenitor cells in analyses of death from cardiovascular causes (P=0.01) and a first major cardiovascular event (P<0.001) (Fig. 1 and 2). Revascularization (P<0.001) and hospitalization

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Total (N=507)	Group 1: Low EPC Level (N=168)	Group 2: Medium EPC Level (N=172)	Group 3: High EPC Level (N=167)	P Value for Trend
Age — yr	66.6±10.8	66.1±11.0	66.3±10.9	67.5±10.5	0.25
Sex — no. (%)					0.67
Female	167 (32.9)	60 (35.7)	51 (29.7)	56 (33.5)	
Male	340 (67.1)	108 (64.3)	121 (70.3)	111 (66.5)	
Cardiovascular risk factors — no. (%)					
Arterial hypertension	432 (85.2)	144 (85.7)	148 (86.0)	140 (83.8)	0.63
Hyperlipidemia	402 (79.3)	123 (73.2)	145 (84.3)	134 (80.2)	0.11
Diabetes	147 (29.0)	56 (33.3)	47 (27.3)	44 (26.3)	0.19
Family history of coronary artery disease	76 (15.0)	30 (17.9)	24 (14.0)	22 (13.2)	0.18
Smoking	116 (22.9)	33 (19.6)	33 (19.2)	50 (29.9)	0.02
Body-mass index ≥25†	336 (66.3)	105 (62.5)	126 (73.3)	105 (62.9)	0.37
Medical history — no. (%)					
Myocardial infarction					0.64
Acute (<24 hr)	17 (3.4)	7 (4.2)	2 (1.2)	8 (4.8)	
Subacute (24 hr–7days)	43 (8.5)	13 (7.7)	11 (6.4)	19 (11.4)	
Previous myocardial infarction	163 (32.1)	58 (34.5)	52 (30.2)	53 (31.7)	
Stroke	41 (8.1)	14 (8.3)	10 (5.8)	17 (10.2)	0.54
Renal insufficiency	91 (17.9)	27 (16.1)	30 (17.4)	34 (20.4)	0.51
Percutaneous coronary intervention					0.19
Previous	182 (35.9)	67 (39.9)	66 (38.4)	49 (29.3)	
Current	227 (44.8)	86 (51.2)	70 (40.7)	71 (42.5)	
Coronary artery disease — no. (%)					0.19
1 Vessel	118 (23.3)	41 (24.4)	42 (24.4)	35 (21.0)	
2 Vessels	134 (26.4)	44 (26.2)	37 (21.5)	53 (31.7)	
3 Vessels	204 (40.2)	72 (42.9)	70 (40.7)	62 (37.1)	
Stenosis <50%	51 (10.1)	11 (6.5)	23 (13.4)	17 (10.2)	
Left ventricular ejection fraction — %	58.8±15.9	60.9±15.7	59.2±15.8	56.1±16.0	0.008
Medication — no. (%)					
ACE inhibitors	281 (55.4)	89 (53.0)	92 (53.5)	100 (59.9)	0.18
Angiotensin-receptor blockers	57 (11.2)	28 (16.7)	14 (8.1)	15 (9.0)	0.03
Beta-blockers	333 (65.7)	107 (63.7)	108 (62.8)	118 (70.7)	0.18
Calcium-channel blockers	84 (16.6)	35 (20.8)	26 (15.1)	23 (13.8)	0.08
Diuretics	215 (42.4)	59 (35.1)	73 (42.4)	83 (49.7)	0.007
Statins	280 (55.2)	82 (48.8)	99 (57.6)	99 (59.3)	0.05
Nitrates	176 (34.7)	62 (36.9)	59 (34.3)	55 (32.9)	0.45
Aspirin	335 (66.1)	104 (61.9)	116 (67.4)	115 (68.9)	0.45
Clopidogrel	113 (22.3)	41 (24.4)	37 (21.5)	35 (21.0)	0.18
Leukocyte count ($\times 10^{-9}$ per liter)	7.44±2.51	7.40±2.38	7.33±2.16	7.79±2.93	0.25

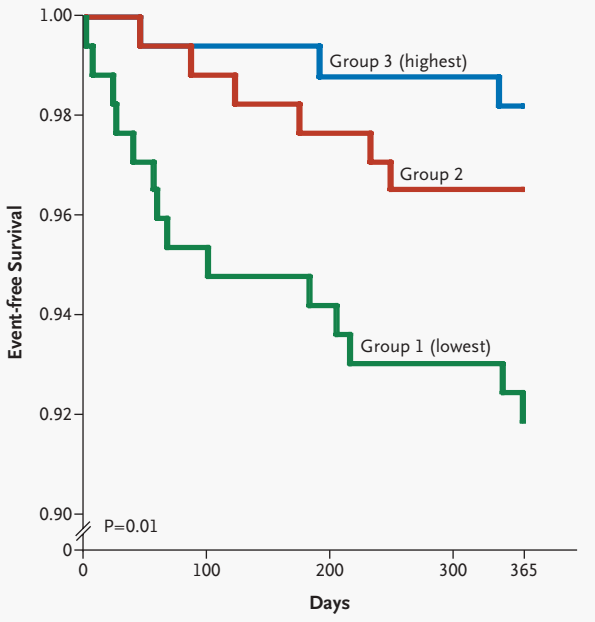
* Plus–minus values are means ±SD. Group 1 represents patients with log numbers of endothelial progenitor cells of 1.71 or less, group 2 patients with numbers between 1.72 and 1.96, and group 3 patients with numbers between 1.97 and 3.02. Data are for the 507 patients who completed 12 months of follow-up. Data on body-mass index were missing for 17 patients, on left ventricular ejection fraction for 32 patients, and on medication for 4 patients. EPC denotes endothelial progenitor cells, and ACE angiotensin-converting enzyme.

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Number of Events at 12 Months of Follow-up.*

Event	Total (N=507)	Group 1: Low EPC Level (N=168)	Group 2: Medium EPC Level (N=172)	Group 3: High EPC Level (N=167)	P Value
			number (percent)		
Death from cardiovascular causes	23 (4.5)	14 (8.3)	6 (3.5)	3 (1.8)	0.01
Myocardial infarction	34 (6.7)	10 (6.0)	14 (8.1)	10 (6.0)	0.64
Revascularization	163 (32.1)	74 (44.0)	45 (26.2)	44 (26.3)	0.001
Hospitalization†	186 (36.7)	80 (47.6)	57 (33.1)	49 (29.3)	0.003
Stroke	17 (3.4)	4 (2.4)	7 (4.1)	6 (3.6)	0.65
Death from any cause	43 (8.5)	17 (10.1)	11 (6.4)	15 (9.0)	0.29

* Group 1 represents patients with log numbers of endothelial progenitor cells of 1.71 or less, group 2 patients with numbers between 1.72 and 1.96, and group 3 patients with numbers between 1.97 and 3.02.
† Hospitalization was due to cardiovascular events, including recurrent angina, congestive heart failure, myocardial infarction, stroke, and arrhythmia.



No. at Risk					
Group 1	168	160	158	156	77
Group 2	172	170	168	166	83
Group 3	167	166	165	165	82
Total	507	496	491	487	242

Figure 1. Cumulative Event-free Survival in an Analysis of Death from Cardiovascular Causes at 12 Months, According to Levels of Circulating CD34+KDR+ Endothelial Progenitor Cells at the Time of Enrollment.

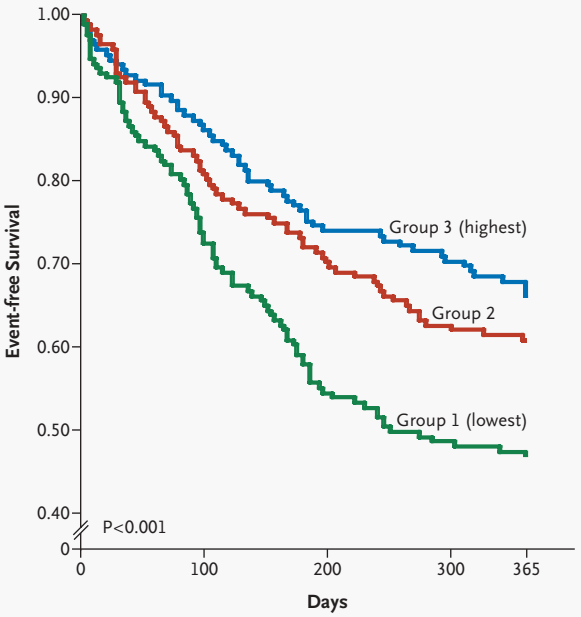
(P=0.001) were significantly more frequent among patients with lower levels of endothelial progenitor cells than among those with higher levels.

Increasing levels of CD34+KDR+ endothelial progenitor cells were associated with a decreased risk of death from cardiovascular causes (Table 3). The risk of death from cardiovascular causes was increased by a factor of more than three among patients with low endothelial progenitor-cell levels, as compared with patients with high levels. After adjustment for age, sex, cardiovascular risk factors, concomitant drug therapy, the severity of coronary artery disease, left ventricular ejection fraction, percutaneous coronary intervention, and a diagnosis of acute coronary syndrome, the association between increasing levels of endothelial progenitors and a decreased risk of death from cardiovascular causes remained significant (P=0.001) (Table 3). Decreasing endothelial progenitor-cell levels were associated with the development of a first major cardiovascular event. A multivariate analysis with adjustment for covariates confirmed a significant association between CD34+KDR+ endothelial progenitor-cell levels and the occurrence of a first major cardiovascular event (hazard ratio, 0.74; 95 percent confidence interval, 0.62 to 0.89; P=0.002) (Table 3). In multivariate analyses, the rates of revascularization and hospitalization due to cardiovascular causes were significantly decreased among patients with

high levels of circulating endothelial progenitor cells (hazard ratio for revascularization, 0.77; 95 percent confidence interval, 0.62 to 0.95; P=0.02; and hazard ratio for hospitalization, 0.76; 95 percent confidence interval, 0.63 to 0.94; P=0.01). No significant association was detected between endothelial progenitor-cell levels and acute myocardial infarction and death from any cause.

Cumulative event-free survival increased in stepwise fashion with increasing baseline CD133+ endothelial progenitor-cell levels in an analysis of death from cardiovascular causes (P=0.03 by the log-rank test), a first major cardiovascular event (P=0.04), and hospitalization (P=0.04) (Fig. 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org). In a multivariate analysis, the association between increasing CD133+ endothelial progenitor-cell levels and reduced risks of a first major cardiovascular event (hazard ratio, 0.81; 95 percent confidence interval, 0.66 to 0.98; P=0.03) and hospitalization (hazard ratio, 0.75; 95 percent confidence interval, 0.61 to 0.93; P=0.007) remained significant.

Cumulative event-free survival increased in stepwise fashion with increasing baseline levels of colony-forming units of endothelial cells in an analysis of a first major cardiovascular event (P=0.03), revascularization (P=0.01), and hospitalization (P=0.01) (Fig. 2 of the Supplementary Appendix). A multivariate analysis confirmed a significant association between increasing numbers of colony-forming units and decreased risks of a first major cardiovascular event (hazard ratio, 0.68; 95 percent confidence interval, 0.49 to 0.96; P=0.03), revascularization (hazard ratio, 0.58; 95 percent confidence interval, 0.38 to 0.88; P=0.01), and hospitalization (hazard ratio, 0.59; 95 percent confidence interval, 0.41 to 0.85; P=0.004).



No. at Risk					
Group 1	168	121	92	82	41
Group 2	172	138	120	107	52
Group 3	167	144	123	117	58
Total	507	403	335	306	151

Figure 2. Cumulative Event-free Survival in Analysis of a First Major Cardiovascular Event (Myocardial Infarction, Hospitalization, Revascularization, or Cardiovascular Death) at 12 Months, According to Levels of Circulating CD34+KDR+ Endothelial Progenitor Cells at the Time of Enrollment.

DISCUSSION

Experimental and clinical studies suggest that there is an evolving role for endothelial progenitor cells in neoangiogenesis and rejuvenation of the endothelial monolayer.[6,12,17] The presence of immature circulating cells in the peripheral blood has been advocated as a marker of an organism's regenerative capacity,[25] and current trials of therapy aim to increase the number of progenitor cells at the site of tissue damage.[19-21] Despite numerous studies, the role of endothelial progenitor cells as a prognostic marker is unclear. Various serum markers have been identified that predict mortality and morbidity due to cardiovascular causes.[26-30] In contrast to the measurement of a single serum marker for the prediction of risk, use of a cellular marker of risk, such as the level of endothelial progenitor cells, unifies the complex interactions of multiple negative factors and may yield a better picture of in vivo mechanisms. In this prospective study, we demonstrated that a single measurement of CD34+KDR+ endothelial progenitor cells is a useful tool to predict cardiovascular outcomes in patients with coronary artery disease. During the observational period of 12 months, a significantly higher incidence of death from cardiovascular causes was observed in patients with low baseline levels of endothelial progenitor cells. The association between these levels and death from cardiovascular causes was independent of the severity of coronary artery disease, a diagnosis of an acute coronary syndrome at the time of enrollment, cardiovascular risk factors, and drug therapy known to influence cardiovascular outcomes.

The occurrence of a first major cardiovascular

Table 3. Multivariate Analysis of the Association between Increasing Levels of CD34+KDR+ Endothelial Progenitor Cells and Outcomes.

Outcome	Unadjusted Hazard Ratio (95% CI)	P Value	Adjusted Hazard Ratio (95% CI)*	P Value
Death from cardiovascular causes	0.45 (0.25–0.81)	0.007	0.31 (0.16–0.63)	0.001
First major cardiovascular event	0.72 (0.61–0.86)	<0.001	0.74 (0.62–0.89)	0.002
Myocardial infarction	1.02 (0.67–1.53)	0.94	1.01 (0.64–1.58)	0.97
Revascularization	0.73 (0.60–0.89)	0.002	0.77 (0.62–0.95)	0.02
Hospitalization	0.74 (0.62–0.88)	0.001	0.76 (0.63–0.94)	0.01
Death from any cause	0.87 (0.60–1.27)	0.46	0.67 (0.43–1.05)	0.15

* For all outcomes, the hazard ratio was adjusted for age; sex; smoking status; the presence of hypertension, diabetes, or hyperlipidemia; the severity of coronary artery disease; the left ventricular ejection fraction; percutaneous coronary intervention; a diagnosis of an acute coronary syndrome at the time of enrollment; and concomitant treatment with an angiotensin-converting–enzyme inhibitor, a beta-blocker, a statin, and aspirin. CI denotes confidence interval.

event (acute myocardial infarction, hospitalization, revascularization, or death from cardiovascular causes) was associated with reduced endothelial progenitor-cell levels. Analyses of the prespecified single end points revealed that hospitalization, the need for revascularization, and to a lesser extent, the rate of death from cardiovascular causes were the major factors for the prediction of end points.

Studies in animals suggest that enhancement of the number of circulating endothelial progenitor cells through exercise training, statin therapy, or estrogen therapy improves the replenishment of the endothelial monolayer by endothelial progenitor cells after vascular injury and — due to enhanced restoration of the endothelial monolayer — diminishes neointima formation.[14,16,31-33] In humans, a small-scale study suggests that there is a higher incidence of restenosis in patients with reduced levels of circulating endothelial progenitor cells than in patients with increased numbers.[34] In our study, we demonstrated that patients with high numbers of endothelial progenitor cells had a reduced risk of revascularization. These findings suggest that endothelial progenitor cells contribute to the restoration of the endothelial monolayer, as suggested by data from experimental studies.

Endothelial progenitor-cell levels were not predictive of death from all causes, acute myocardial infarction, or stroke. This finding may suggest that there was an excess of deaths from noncardiovascular causes among patients with increased endothelial progenitor-cell levels. However, no excess of particular noncardiovascular causes of death were identifiable (data not shown). Small-scale studies suggest that after acute myocardial infarction, the numbers of circulating CD34+ and CD133+KDR+ endothelial progenitor cells are up-regulated in response to tissue ischemia.[3,35] Given the results of our study, we have to assume that the role of endothelial progenitor cells in acute myocardial infarction or stroke is more complex than was initially expected. In our study, endothelial progenitor cells that were mobilized after acute myocardial infarction were functionally impaired (data not shown). This is in accordance with the finding that in patients with congestive heart failure, there is impaired function of progenitor cells.[36] At present, the pathophysiologic consequences of this dysfunction are unknown. Further studies are needed to elucidate the exact role of endothelial progenitor cells in acute myocardial infarction.

In addition to CD34+KDR+ endothelial progenitor cells, we measured the numbers of immature CD133+ cells, which also correlated with cardiovascular outcomes. In order to determine the functional properties of formerly circulating endothelial progenitor cells, we determined the number of endothelial colony-forming units in a subgroup of patients. We confirmed and extended the findings of Hill et al.,[22] demonstrating that functional properties of endothelial progenitor cells influence cardiovascular outcomes.

Our results suggest that circulating endothelial progenitor cells in patients with coronary artery disease can be used to identify patients at high risk for major adverse cardiac events. This finding supports the notion that immature cells play an important part in the pathogenesis of atherosclerotic disease and that the measurement of endothelial progenitor cells may improve risk stratification. Further studies

assessing the therapeutic targeting of circulating endothelial progenitor cells are warranted to prove the underlying biologic concept that endothelial-cell regeneration through circulating endothelial progenitor cells is necessary for vascular healing.

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