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Circulating Endothelial Progenitor Cells as a New Marker of Endothelial Dysfunction or Repair in Acute Stroke

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Background and Purpose—Understanding on distinct subsets of endothelial progenitor cells may provide insights of endothelial dysfunction or repair in the acute ischemic event. Recent in vitro data have reported the colony-forming unit (CFU) and outgrowth cell population as a subset of endothelial progenitor cells. In this study, we undertook to validate the significance of CFU number and outgrowth cell yield in acute stroke.

Methods—Mononuclear cells were isolated from the peripheral blood of 75 patients with acute stroke, 45 patients with chronic stroke, and 40 age-matched healthy volunteers. CFU numbers were counted after culturing them for 7 days, and outgrowth cell appearance was measured during the 2 months of culture. Endothelial progenitor cell function was also evaluated by matrigel plate assays. Independent parameters predicting CFU number and outgrowth cell yield were assessed using logistic regression analysis.

Results—The CFU numbers and tube formation abilities in matrigel assays were significantly reduced in patients with acute stroke compared with patients with chronic stroke or healthy control subjects. Moreover, patients with large artery atherosclerosis had much lower CFU numbers and functional activities than ones with cardioembolism. Outgrowth cells were isolated from 10% of healthy control subjects and 22% of patients with chronic stroke during the cultures, but from 71% of patients with stroke. Multivariate analysis identified glycosylated hemoglobin and National Institutes of Health Stroke Scale on admission as significant independent predictors of a low CFU number and a high isolation frequency of outgrowth cells, respectively.

Conclusion—CFU number may thus represent an accumulated endothelial progenitor cell dysfunctional status, whereas outgrowth cell appearance may reflect the resilience of the systemic circulation to acute ischemic stress. **(*Stroke*. 2008; 39:1441-1447.)**

Key Words: colony-forming unit ■ endothelial progenitor cells ■ outgrowth cells ■ peripheral blood mononuclear cells ■ stroke

Endothelial progenitor cells (EPCs) constitute a circulating pool of cells that counteract ongoing risk factor-induced endothelial cell injury and replace dysfunctional endothelium.[1] Recent studies have demonstrated that an accumulation of cardiovascular risk factors and the presence of coronary, cerebral, or peripheral atherosclerosis are associated with dysfunction and reduced numbers of endothelial progenitor cells.[1–6] Moreover, a low EPC level has been shown to be an independent risk factor of future cardiovascular events.[7,8] On the other hand, EPCs are mobilized from bone marrow during acute ischemia and contribute to the neovascularization of ischemic tissues.[9–13] Limb ischemia and acute myocardial infarction have been associated with a rapid increase in circulating EPC numbers, and vascular traumas such as coronary bypass grafting or burn injury are also suggested as inducing the rapid transient mobilization of EPCs.[11–13] Thus, EPC levels must be carefully interpreted in the combined setting of acute ischemia and chronic vascular insult.

Unlike acute coronary syndrome, which has many nonspecific and specific plasma or serum markers that may be used to both diagnose and assess the severity of myocardial infarction, no similar established markers exist for patients with stroke. Biological assays of EPCs may allow clinicians to demonstrate the specific mechanisms of ischemic lesions and to predict their severities and courses. Furthermore, after cerebral ischemia, neovascularization and new vessel growth

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occur in ischemic areas.[14] New endothelial cells in the setting of ischemia are partly derived from circulating EPCs of bone marrow origin. Thus, the isolation of circulating EPCs may help in the development of EPC-based cell therapies in stroke. However, data on the biology of circulating EPCs at the acute stage in subjects with stroke are sparse. In fact, only one study has reported reduced number of colonies of cultured circulating EPCs in acute and stable patients with stroke.[6]

Our understanding of the growing number of reports on EPC biology has been hampered by a lack of corroborated methods than can precisely identify EPCs. Whereas some investigators have used flow cytometry as a means of estimating EPC levels,[2,7,13] others refer to colony-forming units (CFUs).[1,6] As the number of circulating EPCs represents a dynamic balance between production induced by chronic or acute stimuli and consumption in damaged areas, studies by flow cytometry at limited time points during acute ischemia are of limited usefulness. Emerging evidence indicates that CFU number more appropriately represents EPC functional properties rather than EPC number.[1,2,15] Whereas EPC function is critical for endothelial maintenance and repair, CFU number may indicate a cumulative vascular risk in ischemia. On the other hand, EPCs constitute a heterogenous population with distinct growth characteristics. This problem of heterogeneity is confounded by the different names used, ie, endothelial outgrowth cells, circulating angiogenic cells, and endothelial-like cells, given to possibly overlapping cell types.[16–20] The cell population outgrown from the long-term cultures may be a subset of true EPCs derived from bone marrow with the potential for vascular repair after injury.[18–20] However, to date, no study has validated that outgrowth cell yield is a potential marker of circulating EPC levels. In the present article, a simple yet precise method was used to count CFUs and to observe the appearance of outgrowth cells, which represents a modest first approach in this subject area. In addition, we attempted to identify factors associated with EPC parameters in the setting of acute stroke.

Methods

Study Population

Eligible patients (age, 30 to 70 years) with a clinically and radiologically proven first ischemic stroke and admitted to the Department of Neurology, Seoul National University Hospital were recruited consecutively. As a control group, age- and sex-matched healthy subjects, free from atherothrombotic or hematologic disease, were selected from volunteers. In addition, age- and sex-matched patients with a stroke history (≥3 months before the recruitment) were selected from patients referred to the outpatient clinic of the Department of Neurology. The total study population included 75 patients with acute stroke, 45 patients with chronic stroke, and 40 healthy subjects; and their demographic and medical data were collected as described in the online supplementary methods. The study protocol was approved by the Scientific Review Committee and the Institutional Review Board of Seoul National University Hospital and all enrolled subjects provided written informed consent as required by the Declaration of Helsinki.

Isolation and Culture of Peripheral Blood-Derived Mononuclear Cells

After obtaining informed consent, peripheral blood was sampled from patients 5 to 7 days after symptom onset (6.9±4.7 days). Total human of peripheral blood-derived mononuclear cells (PB-MNCs) were isolated from 50 mL of blood from each of the 160 enrolled subjects by density gradient centrifugation using Histopaque-1077 (Sigma, St Louis, Mo) as described previously[19,20] and online supplementary methods.

Colony-Forming Unit Counts

CFUs were defined as a central core of rounded cells surrounded by elongating and spindle-shaped cells and were counted after 7 days in culture. Cell clusters alone without emerging spindle cells were not counted as positive. Colonies were counted manually in a minimum of 3 wells of each 12-well plate by 2 independent observers who were unaware of clinical profiles, and results were expressed as average numbers of CFUs per well. In selected samples, the endothelial phenotype was confirmed using endothelial-specific indicators, ie, uptake of DiI-LDL, staining for UEA-1 lectin, CD31, VE-cadherin, CD34, and KDR, as previously reported.[16–20]

Matrigel Plate Assay

In selected subjects (n=6 per group), angiogenic activities of conditioned media were assessed using Matrigel plates (BD Biosciences, Bedford, Mass) and human umbilical vein endothelial cells (HUVECs). Conditioned media were obtained by replacing the media of 4-day MNC cultures with serum-free endothelial basal media (EBM; Clonetics, Baltimore, Md) supplemented with EGM-2 single aliquots (growth factors like vascular endothelial growth factor and fibroblast growth factor-2 were omitted), and cells were then cultured for an additional 72 hours. HUVECs grown overnight in EBM were treated with trypsin and resuspended in EBM. The wells of 96-well tissue culture plates were coated with Matrigel (0.04 mL/well), which was left to solidify for 1 hour at 37°C. HUVECs (15 000 in 0.15 mL EBM) were then added to each well. Conditioned media (200 μL) were added to these HUVECs (in sextuplicate) and 16 hours later, media were removed. The cells were then fixed, and the lengths of structures resembling capillaries were measured over whole well areas using Image-Pro Plus (Media Cybernetics, Silver Spring, Md). The total length of tube networks was expressed as mm/mm^2, and the total area of complete tubes formed by cells was expressed as mm^2/mm^2.

Statistical Analysis

All data in this study are expressed as means±SD. The 3 study populations and the stroke subtypes were compared in terms of the following variables: background characteristics (age, gender, National Institutes of Health Stroke Scale [NIHSS], infarct location), risk factors (hypertension, diabetes mellitus, atrial fibrillation, dyslipidemia, smoking), and culture parameters (CFU number, outgrowth cell yield). Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test. Comparisons between groups were analyzed using the 2-tailed unpaired Student t test or analysis of variance followed by Tukey's test for normally distributed variables and using the Kruskal-Wallis test for nonnormally distributed variables. Categorical variables were compared using the Pearson χ^2 test. Correlations between 2 continuous variables were performed using Spearman's rank correlation coefficients. Next, univariate associations between specific parameters and CFU counts and outgrowth cell yields were assessed. The dependent variables were the CFU counts and outgrowth cell appearance, and the independent variables considered were patient age, gender, hypertension, diabetes mellitus, dyslipidemia, smoking status, and a variety of radiological and laboratory values. To identify independent determinants of CFU number and outgrowth cell yield in the patients with acute stroke, multivariate logistic regression analysis was performed for various clinical, radiological, and laboratory parameters. Probability values of <0.05 were regarded as being statistically significant. SPSS software (SPSS 12.0; SPSS Inc, Chicago, Ill) was used for the analyses.

Results

Study Population Demographics

The baseline characteristics of the 160 study subjects are summarized in Table 1. CFU and outgrowth cells were

Table 1. Clinical Characteristics of the Study Population

	Healthy (n=40)	Chronic Stroke (n=45)	Acute Stroke (n=75)	*P* Value
Age	58.4±11.0	61.5±9.6	63.1±11.6	0.763
Sex, male (%)	25 (62.5)	28 (62.2)	48 (63.0)	0.575
Hypertension (%)	0	28 (62.2)	41 (54.7)	0.001*
Diabetes mellitus (%)	0	18 (40.0)	24 (32.4)	0.004*
Dyslipidemia (%)	0	9 (20.0)	14 (18.9)	0.019*
Current smoking (%)	6 (15.0)	16 (35.5)	28 (37.3)	0.106
Atrial fibrillation (%)	0	8 (17.7)	22 (29.7)	0.020*
CFU	72.4±24.5	50.7±27.4	21.3±16.5	0.001*
Isolation of outgrowth cells (%)	4 (10.0)	10 (22.2)	53 (70.7)	0.000*

*$P<0.05$, Pearson χ^2 test or analysis of variance followed by Tukey's test.

derived from PB-MNC cultures (Supplemental Figure I, available online at http://stroke.ahajournals.org). CFU numbers were significantly lower in patients with acute stroke than in patients with chronic stroke and healthy subjects (21.3±16.5 versus 50.7±27.4 and 72.4±24.5, $P=0.001$, analysis of variance). Outgrowth cells were only isolated from 4 (10.0%) and 10 (22.2%) of 40 healthy subjects and 45 subjects with chronic stroke, respectively, but were readily isolated from 53 (70.7%) of the 75 patients with stroke ($P<0.001$, Pearson χ^2 test).

Characteristics of Stroke Subpopulations

Subjects with stroke were divided into 4 subgroups based on stroke etiology. Of the 75 consecutive patients with stroke recruited, 25 (33.3%) patients were allocated to the large artery atherosclerosis (LAA) group, 18 (24.0%) to the cardioembolism (CE) group, 23 to the small vessel occlusion (SVO; 30.7%), and the 9 to the intracerebral hemorrhage (ICH; 12.0%); these groups were compared in terms of the following variables: background characteristics (age, gender, NIHSS), risk factors (hypertension, diabetes mellitus, heart disease, dyslipidemia, smoking), radiological data (lesion volumes), and culture outcome data (CFU, outgrowth cell yield). As shown in Table 2, these 4 groups did not differ with respect to age, gender, dyslipidemia, or smoking. Hypertension was more common in patients with LAA, SVO, and ICH than in those with CE; nearly 60% of patients with LAA and SVO had hypertension. Atrial fibrillation was more prevalent in the CE group. The SVO group had milder neurological deficits and smaller infarct volumes than the other 2 groups, and the CE group had more severe neurological deficits at the time of stroke. We also examined whether these 4 stroke subtypes was associated with culture outcomes, CFU number, and outgrowth cell isolation. The CE group had significantly higher CFU numbers (34.0±13.5) than the LAA (14.5±8.5), SVO (18.3±17.5), and ICH groups (22.5±23.4; $P=0.001$, analysis of variance followed by Tukey's test). Outgrowth cells tended to be more frequently observed in the CE and LAA groups than in the other 2 groups, but this was not significant.

Matrigel Tube Assay in the Ischemic Stroke Subpopulations

To investigate whether the paracrine functions of CFU-derived cells are differentially affected by ischemic stroke subtype, we examined the angiogenic potentials of their conditioned media using Matrigel assays. HUVECs were seeded on Matrigel matrices in the conditioned media of CFU-derived cells from patients with stroke overnight (n=6 for each stroke subtype) or from healthy subjects (n=6). Conditioned media from healthy subjects markedly stimulated tube formation as compared with nonconditioned media

Table 2. Characteristics of the Acute Stroke Subpopulations

	LAA (n=25)	CE (n=18)	SVO (n=23)	ICH (n=9)	*P* Value
Age	60.8±12.2	67.2±11.4	61.1±8.9	67.1±14.8	0.197
Sex, male (%)	17 (68.0)	12 (66.7)	12 (52.2)	7 (77.8)	0.500
Hypertension (%)	14 (56.0)	5 (27.8)	15 (65.2)	7 (77.8)	0.173
Diabetes mellitus (%)	11 (44.0)	3 (16.7)	7 (30.4)	3 (37.5)	0.295
Dyslipidemia (%)	5 (20.0)	3 (16.7)	4 (17.4)	2 (22.2)	0.951
Current smoking (%)	11 (44.0)	4 (22.2)	3 (13.0)	0	0.218
Atrial fibrillation (%)	0	15 (83.3)	2 (8.7)	0	0.000*
CFU	14.5±8.5	34.0±13.5	18.3±17.5	22.5±23.4	0.001*
Isolation of outgrowth cells (%)	18 (72.0)	15 (83.3)	14 (60.9)	6 (66.7)	0.730
Initial NIHSS	4.7±4.5	7.8±6.4	1.3±2.7	5.1±3.7	0.025*
Lesion volume, cm^3	22.2±42.7	26.1±41.5	1.2±0.5	22.3±17.4	0.063

*$P<0.05$, Pearson χ^2 test or analysis of variance followed by Tukey's test.

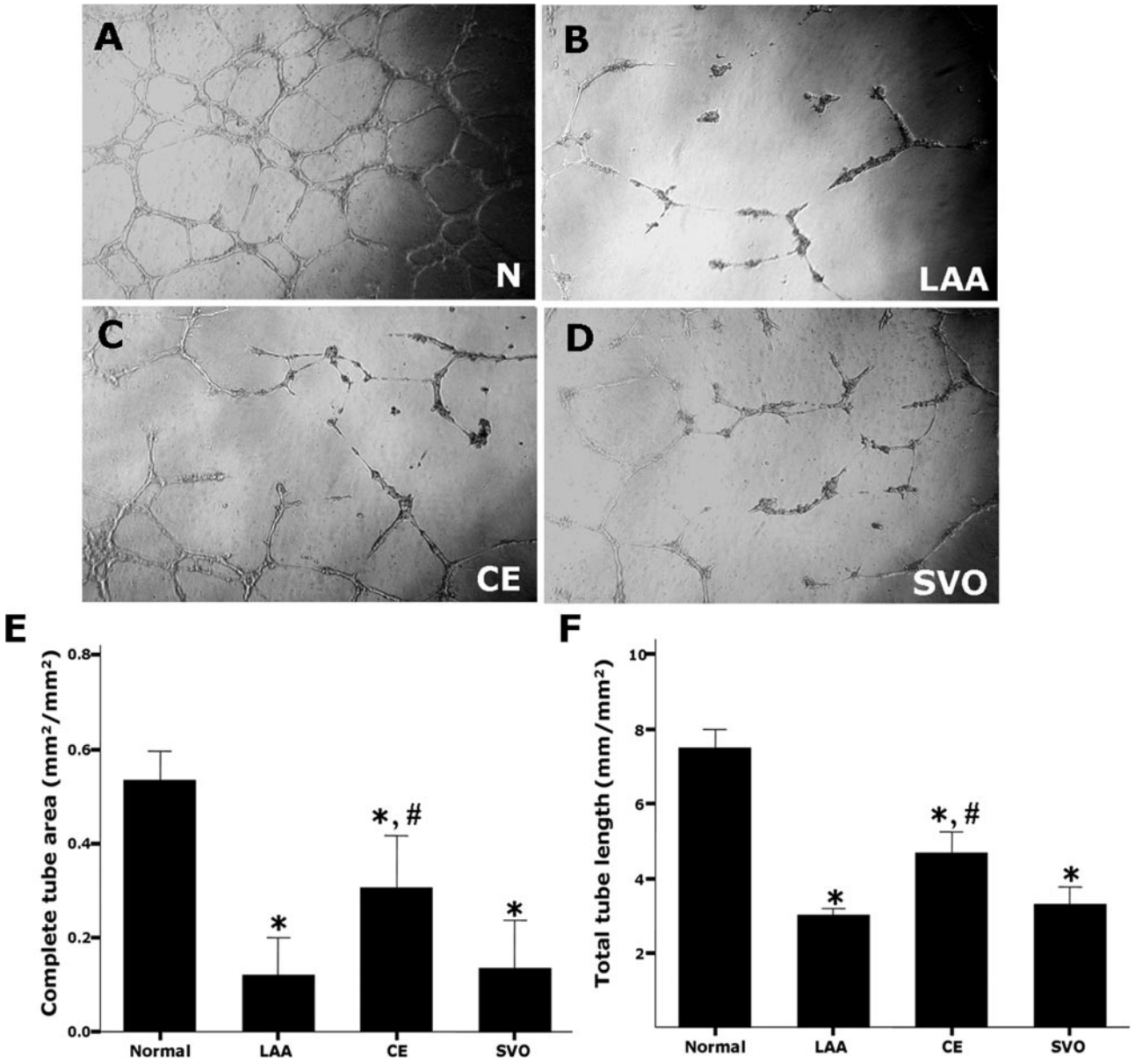

Figure. Matrigel plate assays in the 3 stroke subpopulations. Cultures of PB-MNCs were used to condition media for 3 days, and conditioned media were added to HUVECs on Matrigel-coated 96-well plates. Representative photomicrographs show the formation of tube-like structures in healthy control subjects (A) and 3 distinct stroke subpopulations (B: LAA; C: CE; D: SVO). Complete tube areas (E) and total tube network lengths (F) were determined by Matrigel assays. Results are expressed as means±SD; n=6 for each group; *$P<0.05$ versus healthy control subjects; #$P<0.05$ versus the LAA group.

(EBM), demonstrating that CFU-derived cells can facilitate angiogenesis in a paracrine fashion, or alternatively, that cultured MNCs from patients with stroke secrete factors that impair angiogenesis in vitro (Figure A–D). As shown in Figures E and F, a detailed analysis of CFU-derived cell functionality versus stroke subtype revealed higher values for complete tube area and total tube length in the CE group than in the LAA and SVO groups (complete tube area: 0.31±0.10 versus 0.12±0.07 and 0.13±0.09 mm^2/mm^2; total tube length: 5.70±0.54 versus 4.35±0.67 and 3.98±0.18 mm/mm^2, respectively).

Predictors for Lower Colony-Forming Unit Number in Patients With Acute Stroke

To identify the factors that affect CFU number in the setting of acute stroke, the clinical, radiological, and laboratory parameters were analyzed for correlations with CFU levels and compared in low CFU (≤10 CFU) and intermediate CFU groups (>10 CFU). CFU levels tended to decline linearly with advancing age and higher glycosylated hemoglobin (HbA1c), but this tendency did not reach statistical significance (age: $r=-0.104$, $P=0.180$; HbA1c: $r=-0.179$, $P=0.203$). Age, HbA1c, and homocysteine levels (at admission) were higher in the low CFU group than in the intermediate CFU (age: 65.7±9.9 versus 61.9±12.6; HbA1c: 6.8±1.6 versus 5.8±0.6; homocysteine: 14.5±6.8 versus 9.1±5.7, respectively). No significant differences were found in the initial NIHSS, infarct volumes, lacunes, microbleeds, C-reactive protein, cholesterol, high-density lipoprotein, or low-density lipoprotein between the low CFU and interme-

Table 3. Factors Associated With the CFU Numbers of Patients With Acute Stroke

	Low CFU (n=29)	Intermediate CFU (n=46)	*P* Value
Age	65.7±9.9	61.9±12.6	0.006*
Hypertension (%)	18 (62.1)	23 (50.0)	0.348
Diabetes mellitus (%)	16 (55.2)	29 (17.8)	0.002*
Atrial fibrillation (%)	4 (13.8)	18 (40.0)	0.020*
Dyslipidemia (%)	4 (13.8)	10 (22.2)	0.545
Current smoking (%)	11 (37.9)	17 (37.0)	0.119
Initial NIHSS	5.4±4.5	4.8±4.9	0.629
Infarct volume, cm^3	20.7±42.6	14.2±21.7	0.420
Lacune	3.1±2.5	2.5±1.9	0.205
Microbleed on gradient recalled echo	3.2±4.1	2.9±8.7	0.860
Leukoaraiosis	1.3±1.0	1.2±1.2	0.700
HbA1c, %	6.8±1.6	5.8±0.6	0.008*
C-reactive protein, mg/dL	1.3±2.1	1.6±2.5	0.623
Fibrinogen, mg/dL	360.4±108.2	357.2±97.1	0.900
Vitamin B12, pg/mL	524.5±263.4	625.2±476.1	0.337
Homocysteine, μmol/L	14.5±6.8	9.1±5.7	0.032*
Folate, ng/mL	6.4±6.2	6.4±2.9	0.849
Hematocrit, %	41.8±4.1	40.9±5.6	0.259
Cholesterol, mg/dL	170.5±34.2	184.0±33.9	0.101
Triglyceride, mg/dL	116.6±37.4	139.1±78.0	0.144
High-density lipoprotein, mg/dL	42.9±11.7	41.1±13.4	0.689
Low-density lipoprotein, mg/dL	104.4±31.6	114.5±32.3	0.196

*$P<0.05$, Student *t* test or Pearson χ^2 test.

diate CFU groups (Table 3). By univariate analysis, 4 variables were found to significantly differentiate patients with a low CFU number from those with an intermediate CFU: age ($P=0.006$), diabetes mellitus ($P=0.002$), HbA1c ($P=0.008$), and homocysteine ($P=0.032$). All variables shown in Table 3 were initially entered into a logistic regression model, and the least significant variables were removed from the model in a stepwise fashion. Table 4 shows the adjusted ORs of variables for independently predicting CFU levels. Multivariate analysis showed that only HbA1c significantly and independently predicted CFU numbers.

Predictors of Outgrowth Cell Generation in Patients With Acute Stroke

To identify factors that predict the outgrowth cell appearance in the setting of acute stroke, we categorized patients into 2 groups: outgrowth cell-negative and -positive groups. NIHSS scores, infarct volumes, and C-reactive protein on admission were greater in the outgrowth cell-positive group than in the outgrowth cell-negative group (NIHSS: 2.7±3.0 versus 6.0±5.0, $P=0.005$; infarct volume: 7.5±11.7 versus 22.5±38.8 cm^3, $P=0.029$; C-reactive protein: 0.5±0.8 versus 1.8±2.5, $P=0.044$). However, the 2 groups were similar in terms of age, lacunes, microbleeds, HbA1c, homocysteine, cholesterol, high-density lipoprotein, and low-density lipoprotein levels (Table 5). All variables shown in Table 5 were entered into a logistic regression model, and again, most insignificant variables were removed in a stepwise fashion. Table 6 shows the adjusted ORs of independent variables for predicting outgrowth cell appearance. Multivar-

Table 4. Logistic Regression Findings Based on an Analysis of the Low CFU and Intermediate CFU Groups

	OR	95% CI for OR	*P* Value
Age	0.995	0.915–1.033	0.834
HbA1c	0.548	0.317–0.907	0.032
Homocysteine	0.969	0.838–1.105	0.488

Table 5. Factors Associated With the Outgrowth Cell Generation in the Patients With Acute Stroke

	Outgrowth Cells Negative (n=22)	Outgrowth Cells Positive (n=53)	*P* Value
Age	64.1±14.3	62.4±11.4	0.583
Hypertension (%)	11 (50.0)	30 (56.6)	0.620
Diabetes mellitus (%)	6 (27.3)	18 (34.6)	0.597
Atrial fibrillation (%)	4 (18.2)	18 (34.6)	0.179
Dyslipidemia (%)	4 (18.2)	10 (11.2)	0.916
Current smoking (%)	6 (27.3)	22 (41.5)	0.302
NIHSS on admission	2.7±3.0	6.0±5.0	0.005*
Infarct volume, cm^3	7.5±11.7	22.5±38.8	0.029*
Lacune	1.9±2.2	2.2±2.4	0.599
Microbleed on gradient recalled echo	3.4±6.7	3.4±9.4	0.864
Leukoaraiosis	1.3±1.1	1.2±1.1	0.954
HbA1c, %	6.3±0.7	6.3±1.3	0.948
C-reactive protein, mg/dL	0.5±0.8	1.8±2.5	0.044*
Fibrinogen, mg/dL	360.4±115.2	357.2±96.1	0.456
Vitamin B12, pg/mL	699.7±323.4	341.2±266.0	0.707
Homocysteine, μmol/L	12.5±5.0	11.7±6.2	0.331
Folate, ng/mL	6.0±2.9	6.5±3.9	0.399
Hematocrit, %	42.5±3.7	40.9±5.6	0.204
Cholesterol, mg/dL	184.5±30.0	176.5±36.2	0.329
Triglyceride, mg/dL	153.6±62.4	121.1±42.0	0.159
High-density lipoprotein, mg/dL	41.6±13.4	41.9±12.8	0.813
Low-density lipoprotein, mg/dL	108.2±33.5	111.4±32.9	0.616

*$P<0.05$, Student *t* test.

Table 6. Logistic Regression Findings Based on an Analysis of Patient Outgrowth Cell-Negative and -Positive Groups

	OR	95% CI for OR	*P* Value
Age	0.965	0.921–1.012	0.147
NIHSS on admission	1.289	1.026–1.621	0.029
Infarct volume	1.006	0.974–1.039	0.710
C-reactive protein	1.140	0.831–1.564	0.416

iate analysis identified NIHSS score on admission as the only significant independent predictor of outgrowth cell generation.

Discussion

The present study provides evidence of relationships between the known surrogate markers of chronic vasculopathy, ie, HbA1c and homocysteine, and the CFU level in the patients with acute stroke. In addition, indices of neurological damage, namely NIHSS and infarct volume, were found to be significantly associated with outgrowth cell appearance. Our results indicate that the CFU level and outgrowth cell appearance serve as markers of accumulated vascular risk and response to ongoing tissue damage, respectively.

We found that patients with acute stroke had lower CFUs than healthy subjects and subjects with chronic stroke and that the patients with LAA and SVO had much lower CFUs than patients with CE. Our findings are consistent with a previous report,[6] which showed that EPC counts differ significantly among acute stroke, stable stroke, and normal control subjects. Here, we extend their observations and suggest that CFU analysis provides an additional means of understanding stroke pathophysiology. Endothelial cell injury and endothelial dysfunction are predictors of the risk of vascular events, providing stimuli for the development of atherosclerotic plaque. The EPC dysfunction observed prominently in the LAA subgroup might induce endothelial cell dysfunction and, thus, the progression of vascular disease. Impaired EPC function has also been correlated with a variety of risk factors such as hypertension, diabetes mellitus, and smoking.[1,2] In the present study, a higher prevalence of vascular risk factors in the LAA, SVO, and ICH groups than in the CE group might lead to further reduction on CFU levels.

EPCs are believed to augment neovascularization not only by integrating these cells into newly developing capillaries, but also in a paracrine fashion through the secretion of angiogenic growth factors.[21,22] However, it is not known whether this paracrine effect of EPCs is reduced in stroke. In the present study, we found that the EPCs of patients with stroke were impaired in a paracrine function in in vitro angiogenesis assays. Interestingly, abilities to form tubes were more attenuated in the LAA and SVO groups than in the CE group. Because this angiogenesis assay evaluates multiple growth factors involved in blood vessel growth, our results indicate that stroke is not only associated with the absolute number of CFUs, but also significantly with their paracrine functions, and that this is modulated in a stroke mechanism-dependent manner.

We also compared the effects of clinical, radiological, and laboratory parameters on CFU levels in the subjects with acute stroke dichotomized into low CFU and intermediate CFU number groups. One of the factors that may affect the CFU levels is age.[1,2] We observed an age-dependent change in the CFU levels in the patients with acute stroke, resulting in much lower CFU levels in aged patients. These findings suggest that the functional activities of EPCs induced by vascular insults decline gradually with age. On the other hand, CFU levels were found to be related to diabetes mellitus but not to hypertension, dyslipidemia, or smoking. Hyperglycemia has been reported to be associated with endothelial cell dysfunction and reduced neovascularization in response to tissue ischemia.[23,24] Preclinical and clinical studies have convincingly described the detrimental effects of diabetes on EPC number and function,[4,25,26] and our data confirm the findings of previous reports that diabetes impairs EPC function. This effect of diabetes on EPC may be mediated by an increased consumption of EPCs due to their anchoring to diffusely damaged arteries or to a more central impact on bone marrow. An alternative explanation for the observed association is that the higher HbA1c levels characteristic of diabetes mellitus are associated with lower CFU levels. In a previous study, EPC number was found to be significantly related to HbA1c and blood sugar levels in diabetes, and improved glycemic control was found to significantly increase EPC numbers.[26] In particular, in the present study, HbA1c level was found to be an independent predictor of a low CFU number, which suggests that degree of glycemic dysregulation directly affects EPC function.

Outgrowth cells were reported to arise from an unidentified population during long-term culture.[27] These cells had endothelial characteristics as evidenced by their morphologies and protein and gene expressional patterns.[19,20] In the present study, outgrowth cells were generated from most patients with stroke, but not from the majority of control subjects. Furthermore, indices of neurological damage, namely NIHSS and infarct volume, were found to be significantly associated with outgrowth cell appearance. Several studies have demonstrated after profound injury such as myocardial infarction, coronary artery bypass grafting, or burn injury that the number of circulating EPCs increases significantly.[11–13] It is possible that immediate endothelial or neural damage by stroke might induce a compensatory overproduction of progenitor cells from bone marrow for damage repair.

Patients with a lower CFU number might be more vulnerable to the effects of HbA1c or homocysteine, and more intensive risk factor modification may be required in these patients. However, it remains to be seen whether low CFU levels can predict stroke. In addition, it is unknown whether risk factor modification in patients with low EPC can increase EPC levels. Further study is needed to determine whether interventions that target EPC number or function can reduce the incidence of stroke. On the other hand, the high output of outgrowth cells observed in acute stroke during the present study suggests that circulating EPCs may provide a means of endogenous repair to counteract the effects of acute tissue injury and to replace dysfunctional or damaged endothelium. The isolation and expansion of EPCs might be particularly useful for identifying therapeutic approaches that modify the progression and recurrence of stroke.

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Disclosures

None.

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