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Circulating CD133+ CD34+ Progenitor Cells and Plasma Stromal-derived Factor-1 alpha: Predictive Role in Ischemic Stroke Patients

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Abstract

Circulating progenitor cells and stromal-derived factor-1alpha (SDF-1α) have been suggested to participate in tissue repair following ischemic injury. However, the predictive role of circulating CD133+CD34+ progenitors and plasma SDF-1α in ischemic stroke (IS) patients remains unknown. In this study, we recruited 95 acute IS patients, 40 at-risk subjects and 30 normal subjects. The NIH stroke scale (NIHSS), infarct volume and carotid intima-media thickness (IMT) were determined at day 1 and the modified rankin scale (mRS) of functional outcome was assessed at day 21. The levels of circulating CD133+CD34+ cells and plasma SDF-1α were determined by flow cytometry and enzyme-linked immunosorbent assay, respectively. Our data showed that: 1) The levels of CD133+CD34+ cells were lower in at-risk subjects and IS patients at admission (day 1) when compared to normal controls. 2) The day 1 level of CD133+CD34+ cells varied in IS subgroups and inversely correlated with NIHSS and carotid IMT. The level of SDF-1α inversely correlated with NIHSS and infarct volume. 3) The increment rates of circulating CD133+CD34+ cells and plasma SDF-1α within the first week were correlated. 4) Patients with a higher level of CD133+CD34+ cells at day 7 had a low mRS. The increase rate of CD133+CD34+ cells in the first week was inversely associated with mRS. In conclusion, our findings demonstrate that the circulating CD133+CD34+ progenitor cells and plasma SDF-1α can be used as predictive parameters for IS severity and outcome.

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Disclosures

There is no conflict of interest.

Keywords

progenitor cell; CD133+CD34+ cells; SDF-1 α ; ischemic stroke

Introduction

Ischemic stroke (IS) is a devastating disease and the fourth leading cause of death in the United States. However, the biomarkers for prediction of IS severity and outcome are lacking because of its complex pathogenesis and pathophysiology. Endothelial injury is implicated in the onset and progression of IS¹. Previous studies have suggested that circulating progenitor cells play pivotal roles in promoting endothelium repair and in maintaining vascular homeostasis^{2,3}. Circulating CD133+CD34+ progenitors have been shown to participate in endothelial recovery after stroke onset⁴. The level of circulating CD133+CD34+ progenitors have been reported to negatively associate with the level of neuroinflammatory marker in early IS patients. Nevertheless, there is no study directly addressing the potential of circulating CD133+CD34+ progenitors in predicting the functional severity and clinical outcome of IS patients.

Stromal-derived factor-1 α (SDF-1 α) is recognized as one of the important chemokines participating in the process of tissue repair following ischemic injury⁵. The capacity of SDF-1 α in mobilizing progenitors from bone marrow into circulation has been well documented in previous studies^{6,7}. A recent study in IS patients showed that plasma SDF-1 α positively correlates with the level of circulating CD133+KDR+ cells⁸. However, there is no study investigating the correlation of plasma SDF-1 α with circulating CD133+CD34+ cells. Whether the level of SDF-1 α could be used as a surrogate marker for IS remains unclear.

The aim of the present study was to determine the perspective of circulating CD133+CD34+ progenitor cells and plasma SDF-1 α as predictive parameters for IS.

Materials and Methods

Study Subjects and Enrollment Criteria

This study enrolled 95 acute IS patients, 40 at-risk subjects and 30 normal subjects. Diagnosis of acute IS was made according to the standards established by the World Health Organization (WHO)⁹. All patients admitted to the hospital within 72 hrs after stroke symptom onset were confirmed with cranial magnetic resonance imaging (MRI). Medical history recording, blood sample collection, and conventional exams such as 12-lead ECG, chest radiography, and carotid ultrasound were performed at admission (day 1). IS patients were classified into four subgroups: small artery occlusion (SAO), large artery atherosclerosis (LAA), cardioembolism (CE), and undetermined cause (UE), based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria¹⁰. The age-matched at-risk subjects were recruited from subjects with stroke risk factors such as hypertension, diabetes, smoking and alcohol consumption. The normal control healthy subjects also completed

health examinations including brain MRI, carotid ultrasound, blood tests, etc. The general characteristics of all study subjects are summarized in **Table 1**.

Exclusion criteria of subjects for the study included any of the situations: 1) infectious disease in a previous month, 2) autoimmune or peripheral vascular disease or cancer histories, 3) liver and kidney failure, 4) severe cardiac dysfunction, 5) cerebral infarction or hemorrhage histories (less than 3 months before the recruitment), 6) medications for lipid control or inflammation suppression or immunosuppression.

The research proposal was approved by the ethics committee in the Affiliated Hospital of Guangdong Medical College. Written informed consent was obtained from each participant.

Assessment of Neurological Impairment and Recovery

Acute neurological impairment and short-term outcome were evaluated by using NIH stroke scale (NIHSS) on day 1¹¹ and modified rankin scale (mRS) on day 21¹², respectively. Patients with NIHSS score ≥ 7 and < 7 were assigned into the different groups representing the severity of neurological impairment. The outcome was defined as “good” when mRS was ≤ 2 and as “poor” outcome when mRS was > 2 .

Lesion Volume Measured by Brain MRI

Brain MRI was performed using a 1.5T clinical system (Philips Medical Systems). The lesion volume was measured by using diffusion-weighted imaging (DWI) as previously described¹³. Briefly, the DWI was done by using a multi-slice, single-shot spin-echo EPI sequence. Typical sequence parameters were echo time of 118 msec, matrix size of 128×128 , field view of 260×260 , and 7-mm slice thickness, with no gap between slices and with a set of 20 axial slices covering the whole brain. The volume was calculated by the summation of the slice thickness multiply the total lesion area. The MR diffusion sequence at $b=1000$ was run for 3 times, with diffusion gradients applied in each of the x, y, and z directions. To minimize the effects of diffusion anisotropy, an average of all 3 diffusion directions was calculated to give the trace of the diffusion tensor.

Intima-media Thickness, Plaque and Stenosis of Intracranial Artery Determined by Ultrasonography

The intima-media thickness (IMT) of carotid artery was measured through a standard B-mode ultrasound examination with the use of a grey scale high-resolution color Doppler ultrasound (ATL HDI 5000 scanner Philips, ATL ultrasound, Bothell, WA, USA) equipped with 5–12-MHz linear transducer as previously described¹⁴. All procedures were performed on both sides of two longitudinal images of each internal carotid artery by the same operator. The carotid IMT was determined by measuring the distance between the lumen-intima and media-adventia border of the vascular wall. The most distal 1 cm segments (just proximal to the bulbous dilatation) of both common carotid arteries were scanned on longitudinal plane. The average of two IMT values from each side was used to calculate the mean IMT.

Carotid plaque was detected by using an ultrasonograph method¹⁵. In brief, soft plaque was characterized by irregular plaque morphology, uneven low or moderate echo, smooth and continuous surface. Hard plaque was recognized based on rugged plaque, strong echo, and some with acoustic shadow. Mixed plaque included both soft and hard plaque elements. Soft and mixed plaques were unstable plaques. Carotid stenosis and brain artery stenosis were defined according to the criteria set by American College of Radiology Ultrasound Session¹⁶ and the standard method¹⁷.

All assessments and measurements were performed by a trained neurologist who was blinded to the patient's group and goals of analyses.

Flow Cytometric Analysis of Circulating CD133+CD34+ Progenitor Cells

Peripheral blood (2 ml) was collected from patients at days 1 and 7 after admission, or from at-risk subjects or normal subjects on the examination day. The level of circulating CD133+CD34+ progenitors were determined by flow cytometry as previously described^{8, 18}. In brief, the mononuclear cells were isolated from peripheral blood by gradient density centrifugation, then incubated with 10 µl PE-conjugated anti-human CD133 (Miltenyl Biotech, USA), and 10 µl FITC-conjugated anti-human CD34 (eBioscience, USA) antibodies for 30 min at 4°C in the dark. Isotype matched (IgG) nonspecific antibodies (eBioscience, USA) served as negative controls. After incubation, labeled cells were washed and resuspended with 100 µl of phosphate-buffered saline (PBS) for flow cytometric analysis (Coulter Epics XL_MCL flow cytometer; Beckman Coulter). The flow cytometer was set to acquire 100,000 events/sample. As shown in **Fig 1**, the mononuclear cells were gated (P1). The cells within the gate were used for CD133 and CD34 expressing analysis. CD133+CD34+ cells were shown in the upper-right area (Q2). The level of circulating CD133+CD34+ progenitor cells was expressed as percentage of total mononuclear cells in each sample. The change rate of CD133+CD34+ cells from days 1 to 7 was calculated as: $\text{CD133+CD34+ cells (\%)} = (\text{CD133+CD34+ cells}_{D7} - \text{CD133+CD34+ cells}_{D1}) / \text{CD133+CD34+ cells}_{D1} \times 100\%$.

Enzyme-linked Immunosorbent Assay of Plasma SDF-1α

The level of plasma SDF-1α was measured by enzyme-linked immunosorbent assay (ELISA). Briefly, plasma was centrifuged at 1000 g for 10 min. The supernatant was collected as platelet-poor plasma (PPP). The SDF-1α level in the PPP was detected by using human CXCL12/SDF-1α ELISA kit (USCN Inc., USA). The change rate of SDF-1α from day 1 to 7 was calculated as $\text{SDF-1}\alpha (\%) = (\text{SDF-1}\alpha_{D7} - \text{SDF-1}\alpha_{D1}) / \text{SDF-1}\alpha_{D1} \times 100\%$.

Statistical Analysis

Data was expressed as mean ± SEM. Comparisons for two groups were performed by Student's *t*-test. Multiple comparisons were performed by one- or two-way ANOVA. Comparisons for categorical characteristics were conducted by Chi-square test. Correlation between two variables was analyzed using the Spearman's rank correlation test. Multiple linear regression analysis was used for identifying the main impact factors of the mRS at day

21. SPSS software version 17.0 was used. For all tests, a $P < 0.05$ was considered statistically significant.

Results

General Characteristics of Normal Controls, At-risk Subjects and Stroke Patients

As shown in **Table 1**, there was no significant difference in age, gender, total cholesterol (TC), and high density lipoprotein (HDL) among the three groups. The diastolic blood pressure (DBP), systolic blood pressure (SBP), triglyceride (TG) and low density lipoprotein (LDL) were remarkably higher in IS patients and at-risk subjects compared with those in normal controls.

The Level of Circulating CD133+CD34+ Progenitors Was Reduced in IS Patients and At-risk Subjects, and Varied in Different IS Subgroups

The level of circulating CD133+CD34+ cells at day 1 was significantly lower in IS patients and at-risk subjects than that in normal controls. In addition, the level of circulating CD133+CD34+ cells was higher in the IS group when compared with the at-risk group ($p < 0.05$, $n = 30-95/\text{group}$; **Fig 2-A**). This data suggested that acute ischemic stroke could induce the release of progenitors from bone marrow into circulation. Moreover, IS subgroups displayed a variety in the level of CD133+CD34+ cells. Patients with LAA had the lowest level of CD133+CD34+ cells. The CD133+CD34+ cell levels were similar in CE and UE groups, but they were lower than that in SAO group ($p < 0.05$, $n = 14-33/\text{group}$; **Fig 2-B**).

The Level of Circulating CD133+CD34+ Progenitors Was Low in IS Patients with Carotid Plaque and Was Inversely Correlated with Carotid Remodeling

According to the results of carotid ultrasonography, the stroke patients and at-risk subjects had significantly higher rates of carotid atherosclerosis and plaque, and a higher carotid IMT value when compared to those in normal controls (**Tables 1 and 2**). Among the stroke patients, those with carotid plaque (CP) had a lower level of CD133+CD34+ progenitors than those without CP ($P < 0.05$, $n = 34-61/\text{group}$; **Fig 2-C**). In addition, with the spearman's rank correlation analysis, an inverse correlation was found between the level of circulating CD133+CD34+ cells and the carotid IMT value ($r = -0.35$, $p < 0.01$; **Fig 2-D**).

The Level of Plasma SDF-1 α Negatively Correlated with Lesion Volume and NIHSS in Acute IS

The correlations between plasma SDF-1 α and NIHSS or stroke volume was assessed by Spearman's rank test. The results demonstrated that the acute lesion volume inversely correlated with plasma levels of SDF-1 α on both days 1 and 7 (day 1: $r = -0.36$; day 7: $r = -0.63$, $p < 0.01$; **Fig 3-A-B**), and the NIHSS inversely correlated with plasma SDF-1 α level on day 1 ($r = -0.31$, $p < 0.01$; **Fig 3-C**).

The Day 1 Level of Circulating CD133+CD34+ Progenitors Was Associated with NIHSS, and the Day 7 level and Increment Rate of CD133+CD34+ Progenitors Could be Used as a Predictor for Short-term Outcome

The level of circulating CD133+CD34+ cells at day 1 inversely correlated with NIHSS score ($r = -0.658$, $p < 0.01$; **Fig 4-A**). Consistently, patients in the moderate neurological deficit group (NIHSS ≥ 7) had a lower level of CD133+CD34+ cells than that in the mild neurological deficit group (NIHSS < 7) ($P < 0.05$, $n = 43-52/\text{group}$; **Fig 4-B**). These data suggest that the level of circulating CD133+CD34+ cells on day 1 could reflect the stroke severity in acute phase. In addition, we found there was no significant difference of the levels of CD133+CD34+ cells on day 1 between stroke patients with good outcome (mRS ≤ 2) and those with poor outcome (mRS > 2) ($p > 0.05$, $n = 47-48/\text{group}$). Interestingly, the level of CD133+CD34+ cells on day 7 was significantly higher in the good outcome group as compared to those in the poor outcome group ($p < 0.05$, $n = 47-48/\text{group}$; **Fig 4-C**). Therefore, we further analyzed the association between day 1 CD133+CD34+ cell level (CD133+CD34+ cells_{D1}), day 7 CD133+CD34+ cell level (CD133+CD34+ cells_{D7}), the CD133+CD34+ cell change rate (Δ CD133+CD34+ cells) and the good outcome using multiple linear regression analysis. We found that CD133+CD34+ cells_{D7} and

CD133+CD34+ cells were significant predictors of a better short-term outcome on day 21 after acute IS (**Table 3**).

The Increment rates of Circulating CD133+CD34+ Progenitors and Plasma SDF-1 α were Positively Correlated in Acute IS

As shown in **Fig 5-A**, the day 1 level of plasma SDF-1 α (ng/ml) was significantly higher in IS patients as compared to normal controls ($P < 0.05$, $n = 30-95/\text{group}$), and the level of SDF-1 α on day 7 was higher than that on day 1 in IS patients ($P < 0.01$, $n = 95/\text{group}$). From day 1 to 7, the change rates of SDF-1 α (Δ SDF-1 α) and CD133+CD34+ cells (Δ CD133+CD34+ cells) were strongly correlated ($r = 0.691$, $p < 0.01$, **Fig 5-B**).

Discussion

There are several findings in this clinical study. First, the level of circulating CD133+CD34+ progenitors was lower in IS patients than that in normal controls. Second, the day 1 level of circulating CD133+CD34+ cells varied in different IS subgroups and inversely correlated with NIHSS and IMT, and patients with a higher level of CD133+CD34+ cells on day 7 had a better short-term outcome. Third, the increment rates of CD133+CD34+ cells and plasma SDF-1 α were positively correlated within the week following admission. The increment rates of CD133+CD34+ cells could predict the short-term outcome of IS. The level of SDF-1 α was negatively correlated with the NIHSS and infarct volume. These data well demonstrate the perspectives of using CD133+CD34+ cells and SDF-1 α as biological predictors for IS.

Circulating progenitor cells have been shown to participate in tissue repair¹⁹ and angiogenesis²⁰ in response to ischemic injury. Various combinations of surface antigens including CD34, CD133, KDR, VEGFR2, and CD62E have been used to define the phenotypes of circulating progenitors³. CD133 marker is expressed relatively earlier on

progenitor cells from human bone marrow, where KDR is expressed in the late phase of progenitors²¹. A previous study has demonstrated that reduced circulating CD34+KDR+ cells could contribute to the progression of carotid atherosclerosis²². Later, the level of circulating CD133+CD34+ cells was reported to be negatively associated with the level of neuroinflammatory factors in early IS patients⁴. Although, there is no study directly showing the relationship between circulating CD133+CD34+ cells and IS. In this study, we found that the level of circulating CD133+CD34+ cells was significantly higher in IS patients than that in at-risk subjects, suggesting the mobilization of progenitor cells into systemic circulation in response to ischemic injury. This finding is in agreement with another study showing circulating endothelial progenitors were increased in acute IS²³. In addition, our data shows that there is a lower level of CD133+CD34+ cells in IS and at-risk groups when compared to that in normal subjects. This finding is well supported by our previous animal studies^{18, 24} and other clinical trials^{25, 26} showing a lower level of circulating endothelial progenitors in stroke risk conditions such as hypertension and diabetes.

Another interesting finding is that the level of circulating CD133+CD34+ cells varied among different IS subtypes. The LAA stroke patients had the lowest level of CD133+CD34+ cells. The CE and UE patients had the same level of CD133+CD34+ cells, but both of them were lower as compared to that in SAO group. This variation may depend on different pathogeneses among stroke subgroups. For example, the ultrasonography analysis revealed that LAA patients had the highest prevalence rates of unstable plaque, atherosclerosis and artery stenosis. We also found that the stroke patients with carotid plaques had a significantly lower level of CD133+CD34+ cells than those without carotid plaques. Additionally, we found that the circulating CD133+CD34+ cell level was inversely correlated with the carotid IMT value, which is a sign of atherosclerotic remodeling. These data indicate that the level of circulating CD133+CD34+ cells correlate with the severity of carotid artery injury.

In our study, the level of CD133+CD34+ progenitors inversely correlated with the NIHSS score, which indicated that reduction in circulating CD133+CD34+ cell level can predict the severity of neurological impairment in IS patients. Additionally, a high CD133+CD34+ progenitor level on day 7 and a higher increment rate of it in the first week were associated with a lower mRS at day 21, suggesting the predictive ability of the circulating CD133+CD34+ cells on IS outcome. These data together indicate that both the absolute level of circulating CD133+CD34+ cells and the increment rate of CD133+CD34+ cells are useful in predicting the neurological impairment and outcome of IS.

The SDF-1 α /C-X-C chemokine receptor type 4(CXCR4) axis plays a critical role in progenitor cell mobilization, migration and homing for mediating angiogenesis and tissue repair^{3, 27}. The role of SDF-1 α / CXCR4 axis in cerebral repair following IS has been previously revealed by others²⁷ and us^{3, 28}. In this clinical study, we analyzed the relationship between plasma SDF-1 α level and infarct volume as well as NIHSS score by using the spearman's rank test. We found that there was a positive correlation between the increment rates of CD133+CD34+ cells and SDF-1 α . This data is supported by another clinical study showing the relationship between plasma SDF-1 α and the level of circulating

progenitors determined by colony forming assay²⁹. More importantly, we found that plasma SDF-1 α levels at day 1 and/or day 7 negatively correlated with NIHSS score and the acute infarct volume, suggesting its potential for predicting IS severity. Our findings are also in agreement with a previous study showing that SDF-1 α is correlated with circulating CD133+KDR+, CD34+KDR+ and CD133+CD34+KDR+ progenitors⁸.

In conclusion, the data of this study indicate that circulating CD133+CD34+ progenitor cells and plasma SDF-1 α could be useful parameters for predicting the severity and prognosis of acute IS.

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Reference List

1. Dirnagl U. Pathobiology of injury after stroke: the neurovascular unit and beyond. *Ann N Y Acad Sci.* Sep.2012 1268:21–5. [PubMed: 22994217]
2. Andreou I, Tousoulis D, Tentolouris C, Antoniadis C, Stefanadis C. Potential role of endothelial progenitor cells in the pathophysiology of heart failure: clinical implications and perspectives. *Atherosclerosis.* Dec; 2006 189(2):247–54. [PubMed: 16860805]
3. Zhao YH, Yuan B, Chen J, et al. Endothelial progenitor cells: therapeutic perspective for ischemic stroke. *CNS Neurosci Ther.* Feb; 2013 19(2):67–75. [PubMed: 23230897]
4. Bogoslovsky T, Spatz M, Chaudhry A, et al. Circulating CD133+CD34+ progenitor cells inversely correlate with soluble ICAM-1 in early ischemic stroke patients. *J Transl Med.* 2011; 9:145. [PubMed: 21871109]
5. Sharma M, Afrin F, Satija N, Tripathi RP, Gangenahalli GU. Stromal-derived factor-1/CXCR4 signaling: indispensable role in homing and engraftment of hematopoietic stem cells in bone marrow. *Stem Cells Dev.* Jun; 2011 20(6):933–46. [PubMed: 21186999]
6. Leone AM, Rutella S, Bonanno G, et al. Endogenous G-CSF and CD34+ cell mobilization after acute myocardial infarction. *Int J Cardiol.* Aug 10; 2006 111(2):202–8. [PubMed: 16051386]
7. Stellos K, Bigalke B, Langer H, et al. Expression of stromal-cell-derived factor-1 on circulating platelets is increased in patients with acute coronary syndrome and correlates with the number of CD34+ progenitor cells. *Eur Heart J.* Mar; 2009 30(5):584–93. [PubMed: 19109356]
8. Bogoslovsky T, Spatz M, Chaudhry A, et al. Stromal-derived factor-1[α] correlates with circulating endothelial progenitor cells and with acute lesion volume in stroke patients. *Stroke.* Mar; 2011 42(3):618–25. [PubMed: 21257825]
9. Stroke--1989. Recommendations on stroke prevention, diagnosis, and therapy. Report of the WHO Task Force on Stroke and other Cerebrovascular Disorders. *Stroke.* Oct; 1989 20(10):1407–31. [PubMed: 2799873]
10. Adams HP Jr, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* Jan; 1993 24(1):35–41. [PubMed: 7678184]
11. Hacke W, Albers G, Al-Rawi Y, et al. The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase II MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. *Stroke.* Jan; 2005 36(1):66–73. [PubMed: 15569863]

12. Sulter G, Steen C, De KJ. Use of the Barthel index and modified Rankin scale in acute stroke trials. *Stroke*. Aug; 1999 30(8):1538–41. [PubMed: 10436097]
13. Lovblad KO, Baird AE, Schlaug G, et al. Ischemic lesion volumes in acute stroke by diffusion-weighted magnetic resonance imaging correlate with clinical outcome. *Ann Neurol*. Aug; 1997 42(2):164–70. [PubMed: 9266725]
14. Cobanoglu AK, Gungor O, Kircelli F, et al. Role of asymmetric dimethylarginine in the progression of carotid atherosclerosis in renal transplant patients. *Int Urol Nephrol*. Dec 5.2012
15. Yokoyama T, Miyauchi K. [Carotid artery ultrasound and intravascular ultrasound]. *Nihon Rinsho*. May; 2002 60(5):922–6. [PubMed: 12029994]
16. Grant EG, Benson CB, Moneta GL, et al. Carotid artery stenosis: gray-scale and Doppler US diagnosis--Society of Radiologists in Ultrasound Consensus Conference. *Radiology*. Nov; 2003 229(2):340–6. [PubMed: 14500855]
17. Samuels OB, Joseph GJ, Lynn MJ, Smith HA, Chimowitz MI. A standardized method for measuring intracranial arterial stenosis. *AJNR Am J Neuroradiol*. Apr; 2000 21(4):643–6. [PubMed: 10782772]
18. Chen J, Chen S, Chen Y, et al. Circulating endothelial progenitor cells and cellular membrane microparticles in db/db diabetic mouse: possible implications in cerebral ischemic damage. *Am J Physiol Endocrinol Metab*. Jul; 2011 301(1):E62–E71. [PubMed: 21505143]
19. Du F, Zhou J, Gong R, et al. Endothelial progenitor cells in atherosclerosis. *Front Biosci*. 2012; 17:2327–49.
20. Libby P. Coronary artery injury and the biology of atherosclerosis: inflammation, thrombosis, and stabilization. *Am J Cardiol*. Oct 19; 2000 86(8B):3J–8J.
21. Shantsila E, Watson T, Tse HF, Lip GY. New insights on endothelial progenitor cell subpopulations and their angiogenic properties. *J Am Coll Cardiol*. Feb 12; 2008 51(6):669–71. [PubMed: 18261687]
22. Lau KK, Chan YH, Yiu KH, et al. Burden of carotid atherosclerosis in patients with stroke: relationships with circulating endothelial progenitor cells and hypertension. *J Hum Hypertens*. Jun; 2007 21(6):445–51. [PubMed: 17361211]
23. Yip HK, Chang LT, Chang WN, et al. Level and value of circulating endothelial progenitor cells in patients after acute ischemic stroke. *Stroke*. Jan; 2008 39(1):69–74. [PubMed: 18063830]
24. Chen J, Xiao X, Chen S, et al. Angiotensin-converting enzyme 2 priming enhances the function of endothelial progenitor cells and their therapeutic efficacy. *Hypertension*. Mar; 2013 61(3):681–9. [PubMed: 23266545]
25. Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res*. Jul 6; 2001 89(1):E1–E7. [PubMed: 11440984]
26. Tepper OM, Galiano RD, Capla JM, et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation*. Nov 26; 2002 106(22):2781–6. [PubMed: 12451003]
27. Shyu WC, Lin SZ, Yen PS, et al. Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. *J Pharmacol Exp Ther*. Feb; 2008 324(2):834–49. [PubMed: 18029549]
28. Chen J, Chen J, Chen S, et al. Transfusion of CXCR4-primed endothelial progenitor cells reduces cerebral ischemic damage and promotes repair in db/db diabetic mice. *PLoS One*. 2012; 7(11):e50105. [PubMed: 23185548]
29. Sobrino T, Perez-Mato M, Brea D, Rodriguez-Yanez M, Blanco M, Castillo J. Temporal profile of molecular signatures associated with circulating endothelial progenitor cells in human ischemic stroke. *J Neurosci Res*. Sep; 2012 90(9):1788–93. [PubMed: 22513751]

Figure 1. Flow cytometry analysis of circulating CD133+CD34+ progenitors

A, the mononuclear cell population was identified in forward and side scatter plots and gated (P1). B, the cells within the gate were used for CD133 and CD34 expressing analysis. CD133+CD34+ cells were shown in the upper-right area (Q2).

Fig 2. The level of circulating CD133+CD34+ progenitors was reduced in IS and at-risk subjects, varied in different IS subgroups, low in IS with carotid plaque, and inversely correlated with carotid remodeling

A, the day 1 level of CD133+CD34+ cells in normal controls, at-risk subjects and acute IS patients. B, the day 1 level of CD133+CD34+ cells in four stroke subgroups. C, the day 1 level of CD133+CD34+ cells in the patients with (w/) and without (w/o) carotid plaque (CP). D, the correlation between CD133+CD34+ cell level at day 1 and IMT in acute IS patients. Data was expressed as: mean $\pm$ SEM.

Fig 3. The plasma concentration of SDF-1α negatively correlated with the infarct volume and NIHSS in acute IS patients

A-B, the plasma levels of SDF-1α at both days 1 and 7 negatively correlated with the stroke volume. C. the plasma levels of SDF-1α at day 1 inversely correlated with NIHSS. Data was expressed as: mean $\pm$ SEM.

Fig 4. The level of circulating CD133+CD34+ cells correlated with NIHSS and predicts the IS prognosis

A, correlation between CD133+CD34+ cell level on day 1 and NIHSS on admission in stroke patients. B, the day 1 level of CD133+CD34+ cells was much lower in patients with $\text{NIHSS} \geq 7$ than that in patients with $\text{NIHSS} < 7$. C, the patients with a higher CD133+CD34+ level on day 7 after admission had a better outcome ($\text{mRS} \leq 2$) on day 21. Data was expressed as: mean $\pm$ SEM.

Fig 5. The plasma concentration of SDF-1α was increased following ischemic stroke, and its increment rate correlated with the level of circulating CD133+CD34+ cells

A, the level of SDF-1α was increased in stroke patients over the time. B, the increment rate of SDF-1α correlated with the increment rate of CD133+CD34+ cells. Data was expressed as: mean $\pm$ SEM.

Table 1

General characteristics in normal controls, at-risk subjects and stroke patients.

	Normal controls (n = 30)	At-risk Subjects (n = 40)	Stroke patients (n = 95)
Demographics			
Age (yr)	67.43 ± 1.53	67.75 ± 1.54	68.9 ± 1.08
Gender, % male (n)	43.3 (13)	45 (18)	47.4 (45)
Risk factors			
Hypertension, % (n)	----	57.5 (23)	62.1 (59)
Diabetes, % (n)	----	27.5 (11)	28.42 (27)
Smoking, % (n)	----	10 (4)	11.57 (11)
Alcohol consumption, % (n)	3.33 (1)	12.5 (5)	13.68 (13)
Stroke history, % (n)	----	20 (8)	17.89 (17)
Blood pressures			
SBP (mmHg)	109.6 ± 1.65	139.84 ± 2.86 *	144.6 ± 1.96 *
DBP (mmHg)	66.34 ± 1.14	83.5 ± 1.5 *	85.02 ± 1.4 *
Plasma lipids			
TC (mmol/l)	4.46 ± 0.11	4.52 ± 0.15	4.68 ± 0.08
TG (mmol/l)	1.25 ± 0.07	1.53 ± 0.11 *	1.72 ± 0.09 *
HDL (mmol/l)	1.17 ± 0.06	1.18 ± 0.06	1.22 ± 0.01
LDL (mmol/l)	2.42 ± 0.07	2.89 ± 0.15 *	2.96 ± 0.07 *
Ultrasound parameters			
IMT (mm)	0.08 ± 0.002	0.12 ± 0.005 *	0.12 ± 0.003 *
Carotid atherosclerosis, % (n)	0.033 (1)	70 (21) *	66.31 (66) *
Plaque, % (n)	0.066 (2)	42.5 (17) *	64.21 (61) *
Stroke subgroups			
NIHSS 7, % (n)	----	----	45.3 (43)
LAA, % (n)	----	----	31.6 (30)
SAO, % (n)	----	----	34.7 (33)
CE, % (n)	----	----	18.9 (18)
UE, % (n)	----	----	14.7 (14)

Results were expressed as means ± SEM.

* P < 0.05, vs. normal controls; Abbreviations: LAA, large artery atherosclerosis; SAO, small artery occlusion; CE, cardioembolism; UE, undetermined cause; SBP, systolic blood pressure; DBP, diastolic blood pressure ; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, Low density lipoprotein ; IMT, intima-media thickness.

Table 2

Comparison of artery lesion in different stroke subgroups.

	SAO (n=33)	LAA (n=30)	CE (n=18)	UE (n=14)
Atherosclerosis , % (n)	51.51 (17)	100 (30) *	55.55 (10)	78.57 (11)
Unstable plaque, % (n)	12.12 (4)	50 (15) *	0	35.71(5)
Artery stenosis, % (n)	0	100 (30) *	0	28.57 (4)

* P<0.05, vs. SAO or CE or UE. Abbreviations: LAA, large artery atherosclerosis; SAO, small artery occlusion; CE, cardioembolism; UE, undetermined cause. Unstable plaque including soft plaque and mixed plaque.

Table 3

Multiple linear regression analysis of predictors for mRS 2 on day 21 after admission.

Model	B	SE	Beta	t	P
CD133+CD34+ cells _{D1}	1.387	1.262	0.268	1.099	0.275
CD133+CD34+ cells _{D7}	-1.9	0.954	-0.408	-1.991	0.049
CD133+CD34+ cells (%)	-0.451	0.139	-0.423	-3.242	0.002