Original Paper

European Neurology

Eur Neurol 2008;59:38–43
DOI: 10.1159/000109259

Received: October 4, 2006
Accepted: April 11, 2007
Published online: October 4, 2007

Relationship between Detectability of Ischemic Lesions by Diffusion-Weighted Imaging and Embolic Sources in Transient Ischemic Attacks

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Key Words
Diffusion-weighted imaging · Embolic sources · Transient ischemic attacks

Abstract
Background/Aims: The aim of this study is to clarify the relationship between lesion detectability by diffusion-weighted magnetic resonance imaging (DWI) and the etiology of transient ischemic attacks (TIAs). ***Methods:*** A retrospective study was performed on 72 patients with carotid TIAs who underwent DWI studies within 2 weeks after the last episode. ***Results:*** Lesions were detected in 24 of 72 patients (33%). The detectability of lesions was 12% (3/25) in the large-artery atherosclerosis (LA) group, 57% (8/14) in the cardioembolism (CE) group, 8% (1/13) in the small-artery occlusion (SA) group, and 60% (12/20) in the other etiology or undetermined etiology (UD) group. Detectabilities in the CE group and the UD group were higher than those in the LA and SA groups. Of 24 patients with DWI-positive lesions, 17 (71%) had embolic sources in the heart; 9 were classified in the UD group because they had embolic sources both in the heart and large artery. ***Conclusion:*** Ischemic DWI lesions in TIAs are most likely caused by a cardioembolic mechanism. In TIA patients showing lesions on DWI, heart disease should be surveyed as the possible embolic source.

Introduction

According to the Ad Hoc Committee on classification of cerebrovascular disease of 1975, transient ischemic attacks (TIAs) are defined as ischemic cerebrovascular disease in which focal cerebral dysfunction resolves within 24 h [1]. It has been known for years that CT or T_1- and T_2-weighted magnetic resonance imaging (MRI) may occasionally depict small ischemic lesions in TIA patients [2–7]. More recently, diffusion-weighted imaging (DWI) has made it possible to demonstrate ischemic lesions in TIA patients with relatively high frequency [8]. Several studies have shown that the detectability of DWI lesions in TIA patients increases in correlation with the duration of TIA symptoms. However, clarification as to whether the detectability of DWI lesions is related to the etiology of TIA is still lacking. In the majority of previous studies reporting DWI detectability of TIA lesions, both types of TIAs – in the carotid artery territory and the vertebral artery territory – were included indiscriminately. This makes accurate evaluation of TIA duration difficult, since onset and end of symptoms are often obscure in a vertebral artery territory TIA. Therefore, we included only patients with carotid TIA in the present study, examining the relationship between the etiology of TIA and the detectability of DWI lesions.

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Subjects and Methods

A retrospective analysis was performed on 72 patients with carotid TIA who were admitted to our department during the interval from May 1998 to July 2005, and who underwent DWI studies within 14 days of symptom onset after the last TIA. Fifty-two patients were male. The mean (± SD) age of patients was 69 ± 10 years. Patients with isolated amaurosis fugax were excluded. MRI was performed using a Siemens Magnetom Vision 1.5-tesla MR unit. DWI scanning was performed with a single-shot, multislice spin echo and echo planar imaging sequence. DWI parameters comprised: TE = 123 ms; FOV = 23 × 23 cm; matrix = 128 × 200, and slice thickness = 4 mm. Diffusion gradients were applied in the through-plane direction with a b value of 1,100 s/mm^2. Since 1999, imaging parameters have been changed to TE = 100 ms and matrix = 98 × 128. Diffusion gradients were applied in each x, y, and z direction with b values of 1,000 s/mm^2, and trace imaging was calculated. Conventional MRI studies included T_1-weighted (TR/TE: 630/14) and T_2-weighted (TR/TE: 5,400/99) images, and fluid attenuation inversion recovery (TR/TE/TE: 9,000/105/2,400) images were obtained when required.

We assessed whether each patient had ischemic lesions and/or arterial disease compatible with symptoms by reviewing DWI films and the results of conventional cerebral angiography, MR angiography, and carotid ultrasonography. We assessed the presence or absence of cardiac embolic sources based on 12-lead ECG findings, transthoracic and/or transesophageal echocardiography and, when required, 24-hour ECG monitoring. Referring to the TOAST classification [9], all patients were classified into four groups: large-artery atherosclerosis (LA) group, cardioembolism (CE) group, small-artery occlusion (SA) group, and other etiology or undetermined etiology (UD) group. The LA group included patients with more than 50% stenosis of intracranial or extracranial large arteries or with a complicated lesion of more than 3.5 mm in the aortic arch based on findings of conventional cerebral angiography, MR angiography, carotid ultrasonography and transesophageal echocardiography. Patients in this group should not have had significant heart disease. The CE group included patients with significant heart disease that can become an embolic source, such as mechanical prosthetic valves, mitral stenosis with atrial fibrillation, atrial fibrillation, left atrial/atrial appendage thrombus, sick sinus syndrome, recent myocardial infarction within 4 weeks prior to the study, left ventricular thrombus, dilated cardiomyopathy, akinetic left ventricular segment, atrial myxoma, infective endocarditis or patent foramen ovale with peripheral thrombus but without LA. The SA group included patients who had neither significant heart disease nor LA, nor other evidence of disease. The TIA symptoms in this group should have corresponded to any of the traditional clinical lacunar syndromes and should not have been associated with cortical symptoms. The UD group included patients who could not have been classified into other groups because of the following reasons: (1) they had other causes of cerebral ischemia, such as dissection of cervical/cranial arteries, vasculitis or hypercoagulopathy, (2) they had cortical symptoms in spite of an absence of association with significant heart disease, large-artery lesions or evidence of other diseases, and (3) they had both significant heart disease and more than 50% stenosis in a large artery or aortic complicated lesions greater than 3.5 mm. This classification was made by mutual agreement by three neurologists. We also examined the duration of TIA symptoms and the time from onset of TIA to DWI in each patient. Furthermore, we reviewed the correlation between these factors and the detectability of lesions. We also studied whether patients had risk factors for atherosclerosis such as hypertension, diabetes mellitus, hyperlipidemia and smoking, and whether they had a history of cerebral infarction.

Statistical Analysis

Statistical analysis was performed using a commercially available software package (Statview, version 5, SAS Institute Inc., Cary, N.C., USA). Data were expressed as means ± SD. The level of $p < 0.05$ was determined to indicate statistical significance. We statistically compared the four groups as classified above using one-way factorial ANOVA or the Kruskal-Wallis test.

The table of baseline patient characteristics was analyzed using the Yates corrected χ^2 or Fisher test, as appropriate.

Results

Twenty-four of 72 patients (33%) had small ischemic lesions on DWI. There was no significant difference in baseline characteristics between patients with positive DWI lesions and those with negative DWI lesions (table 1). As shown in table 2, the duration of symptoms was significantly longer in patients with positive DWI lesions (4.0 ± 5.1 h) than in those with negative DWI lesions (1.4 ± 2.5 h) ($p < 0.01$). The time from TIA onset to DWI study was also significantly longer in patients with positive lesions (4.5 ± 4.1 days) than in those with negative lesions (2.0 ± 3.2 days) ($p < 0.01$). The detectability of lesions increased in correlation with the duration of TIA symptoms, as shown in figure 1. The detectability of lesions was also influenced by time from TIA onset to DWI, as follows: detectability was 14% (4/29) in the group undergoing DWI at 0–12 h after TIA, 33% (5/15) in the group undergoing DWI at 12–24 h after TIA, 43% (3/7) in the group undergoing DWI at 1–3 days after TIA, 60% (6/10) in the group undergoing DWI at 3–7 days after TIA, 57% (4/7) in the group undergoing DWI at 7–10 days after TIA, and 50% (2/4) in the group undergoing DWI at 10–14 days after TIA. Thus, the detectability of lesions was somewhat lower in patients undergoing DWI within 24 h after TIA than in those undergoing DWI more than 24 h after TIA.

Cerebral angiography was carried out on 25 patients, MR angiography on 58 patients, carotid ultrasonography on 71 patients, transthoracic echocardiography on 58 patients and transesophageal echocardiography on 60 patients. The type of etiology was classified as LA group, 25 patients; CE group, 14 patients; SA group, 13 patients, and UD group, 20 patients. The breakdown of the 20 patients

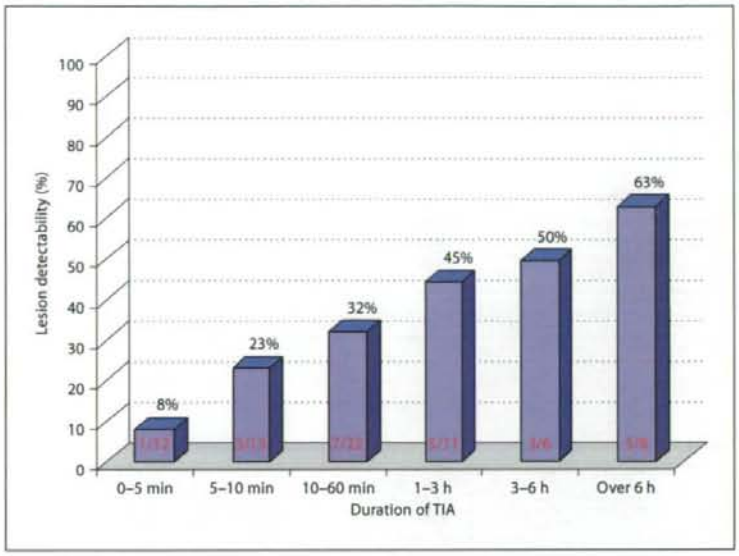

Fig. 1. The duration of TIA symptoms and DWI lesion detectability. The DWI lesion detectability increases in correlation with the duration of TIA symptoms.

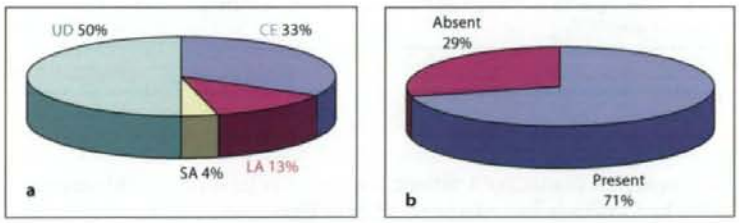

Fig. 2. Details of 24 TIA patients with DWI-positive lesions. **a** 83% of DWI-positive patients belong to the CE or UD groups. **b** 71% of DWI-positive patients have heart disease, either independently or in association with other etiologies.

in the UD group was as follows: (1) 1 patient with both antiphospholipid antibody syndrome and a significant lesion in a large artery, and 1 other patient with cervicocranial dissection and significant atherosclerotic lesions in a large artery, (2) 2 patients with cortical symptoms in association with no abnormality in the heart, large artery and other tests, and (3) 16 patients with both significant heart disease and significant stenotic lesions in large cerebral arteries and/or aorta. As shown in table 3, the detectabilities of lesions in the CE group (57%) and the UD group (60%) were significantly higher as compared with those in the LA group (12%) and the SA group (8%).

The duration of symptoms in the CE group was somewhat longer than in the other three groups, although the difference was not significant (table 3). Time from TIA onset to DWI was somewhat longer in the CE and UD groups as compared with the other two groups. However, results of one-way factorial ANOVA indicated that there was no significant difference in time from TIA to DWI studies between the groups (table 3). DWI studies were performed within 24 h after TIA in 39 patients and more than 24 h after TIA in 33 patients. Percentages of patients undergoing DWI studies within 24 h after TIA were 64% (16/25) in the LA group, 50% (7/14) in the CE group, 62% (8/13) in the SA group, and 40% (8/20) in the UD group. There were no significant differences in the frequency of early DWI studies between the four groups (Kruskal-Wallis test). The CE and UD groups had higher lesion detectability irrespective of time from TIA to DWI studies.

A total of 14 patients had multiple lesions on DWI. The frequency of multiple lesions in each group was as fol-

Table 1. Baseline patient characteristics

	DWI positive (n = 24)	DWI negative (n = 48)
Age, years	68 ± 10	69 ± 10
Male gender	18 (75)	34 (71)
Hypertension	15 (63)	34 (71)
Diabetes mellitus	4 (17)	10 (21)
Hypercholesterolemia	9 (38)	21 (44)
Smoking	13 (54)	31 (65)
History of stroke	1 (4)	8 (17)

Figures in parentheses indicate percentages.

Table 2. Duration of symptoms and time to MRI studies in DWI-positive and DWI-negative patients

	DWI positive	DWI negative
Duration of symptoms, h	4.0 ± 5.1*	1.4 ± 2.5
Time from TIA to MRI, days	4.5 ± 4.1*	2.0 ± 3.2

* $p < 0.01$: significantly longer as compared with the diffusion-negative patients.

Table 3. Comparison of four TIA groups

	LA (n = 25)	CE (n = 14)	SA (n = 13)	UD (n = 20)
Positive DWI lesions	3 (12)	8 (57)[1]	1 (8)	12 (60)[1]
Duration of symptoms, h	1.5 ± 3.0	3.8 ± 5.2	1.7 ± 2.8	2.5 ± 3.9
Time from TIA to DWI, days	1.8 ± 2.6	3.6 ± 4.1	2.6 ± 4.5	3.6 ± 4.1

Figures in parentheses indicate percentages.
[1] The frequency of DWI-positive lesions is significantly higher in the CE and the UD groups as compared with the LA and SA groups ($p < 0.05$).

Table 4. The type of heart diseases and lesion detectability in the CE group

Type of heart diseases	DWI positive	DWI negative
Atrial fibrillation	5	2
Mechanical prosthetic valve	1	1
Akinetic left ventricular segment	2	0
Sick sinus syndrome	0	1
Patent foramen ovale with peripheral thrombus	0	2

lows: in the LA group, 2 of 3 patients with positive lesions (67%) had multiple lesions; the number of lesions was 3 and 5, respectively. In the CE group, 3 of 8 patients (38%) had multiple lesions; the number of lesions was 3 in 2 cases and 4 in the other. In the SA group, 1 positive patient had only a single lesion (0%). In the UD group, 9 of 12 patients (75%) had multiple lesions; the number of lesions was 2 in 5 cases, 3 in 3 cases and 6 in the remainder. Thus, no remarkable relationship was observed between the number of lesions and the etiology.

Of all 24 patients with ischemic lesions on DWI, 8 patients (33%) belonged to the CE group, 12 patients (50%) to the UD group, 3 patients (13%) to the LA group, and 1 patient (4%) to the SA group (fig. 2). Of the 12 patients with positive DWI lesions who belonged to the UD group, 9 had significant heart disease in association with significant large-artery lesions. Thus, of all 24 patients with ischemic lesions on DWI, 17 patients (71%) had significant heart disease either independently or concomitantly with large-artery lesions (fig. 2). In the CE group, 5 of 7 patients with atrial fibrillation had positive DWI lesions (table 4).

Discussion

In recent years, quite a few studies have reported the presence of ischemic lesions on DWI following TIA. In these previous studies, the detectability of TIA lesions on DWI ranged from 20 to 70% [8, 10–16]. Detectability in the present study was 33%, showing a somewhat lower value as compared with previous studies. This may be partly related to the difference in the timing of DWI examinations between the present study and the previous studies. In the present study, approximately 54% of patients underwent DWI within 24 h after TIA onset; the detectability in these patients was low, at 21%. On the other hand, in most previous studies the majority of TIA patients underwent DWI examinations more than 24 h after TIA onset. When we calculated only the detectability of lesions in our patients undergoing DWI more than 24 h after TIA onset, the detectability increased to 48%, showing similar values to those reported in previous studies. The above-mentioned reasoning can also be inferred from the study of Rovira et al. [10]. In their study,

only 9% of patients underwent DWI within 48 h after TIA, and the detectability of lesions in the entire group showed a high value, reaching 67%. In a transient ischemia experiment using rats, the value of the average apparent diffusion coefficient decreased significantly during the ischemic period and then normalized at 60–90 min after ischemia, followed again subsequently by a significant reduction more than 12 h after ischemia [17]. As confirmed in the above experiment, the detectability of lesions on DWI may decrease for a while after a short period of transient cerebral ischemia, and may increase thereafter, although the mechanisms remain unclear. Kidwell et al. [8] first pointed out that the detectability of lesions on DWI in patients with TIA increases in correlation with the duration of symptoms. Since then, similar results have been reported by several authors. In the study by Rovira et al. [10], the detectability of lesions in TIA patients with symptoms lasting less than 6 h was 59%, whereas the value was 100% in patients with symptoms lasting more than 6 h. Crisostomo et al. [11] reported that the detectability of lesions in patients with symptoms lasting more than 1 h was significantly higher as compared with patients with symptoms lasting less than 1 h. Inatomi et al. [12] also reported that the detectability of lesions was significantly higher in TIA patients with symptoms lasting more than 30 min than in those with symptoms lasting less than 30 min. In our study, the detectability of lesions also tended to increase according to the increase in TIA duration.

Previously, few workers performed detailed investigations on the relationship between the detectability of DWI lesions and the etiology of TIA. Rovira et al. [10] reported that the detectability of DWI lesions was higher in TIA patients with large-artery lesions than in those with cardiac lesions. However, the report lacks credibility, since the number of patients in their study was small; only 4 patients had cardiac lesions, whereas 19 patients had large-artery lesions. Nakamura et al. [16] reported higher detectability of DWI lesions in TIA patients with atrial fibrillation as compared with those without atrial fibrillation. However, they focused only on atrial fibrillation and did not clarify DWI detectability in TIA patients without atrial fibrillation who had other types of heart diseases. In the present study, the presence or absence of large-artery lesions and/or cardiac disease was surveyed in a retrospective manner reviewing the results of conventional cerebral angiography, MR angiography, carotid ultrasonography, 12-lead ECG, transthoracic or transesophageal echocardiography, and 24-hour ECG monitoring. The patients were then classified into four groups according to the etiology of TIA, such as LA, CE, SA and UD groups. The results indicated that the detectability of lesions in the CE group and the UD group was higher than that in the other groups. Time from TIA to DWI studies was almost the same in the four groups. Lesion detectability was higher in the CE and UD groups than in the other groups, even when the comparison among the subgroups undergoing DWI studies had been made within 24 h after TIA onset. Therefore, the higher detectability in the CE and UD groups is unrelated to time from TIA to DWI studies. The mean duration of TIA symptoms in the CE group was more than 3 h, which was the longest of all the groups. In general, cardioembolic stroke produces severer symptoms than artery-to-artery embolic stroke. This may be attributable to the fact that emboli originating in the heart tend to occlude larger blood vessels for longer durations as compared with artery-to-artery emboli. This is probably also true in cases of TIA. Microemboli originating in the heart likely occlude larger blood vessels for longer durations as compared with artery-to-artery microemboli. Accordingly, ischemic duration may be longer in TIA patients with heart disease than in those with other types of etiology, and ischemic lesions may be larger in TIA patients with heart disease than in those with other types of etiology. Probably for such reasons, ischemic lesions in cardioembolic TIA may be more readily found on DWI than those in other types of TIA. In the present study, 16 of 20 patients in the UD group had heart disease, and 9 had ischemic lesions on DWI. In these 9 patients, TIA was most likely caused by a cardioembolic mechanism rather than another type of etiology. Johnston et al. [18] conducted a follow-up study in 1,707 patients with TIA for 90 days. In their study, 10.5% of patients developed cardioembolic stroke during the follow-up period, and approximately half of them had stroke within 48 h after TIA. Thus, cardioembolic stroke may occur soon after TIA at a considerably high frequency. A DWI study is considered useful to evaluate etiological mechanisms of TIA. If ischemic lesions are detected on DWI, the presence of heart disease should be suspected, and appropriate medication should be considered to prevent cardioembolic stroke.

Acknowledgement

This work was supported by a Research Grant for Cardiovascular Diseases (18C-2) funded by the Japanese Ministry of Health and Labor.

References

1 Ad Hoc Committee on Cerebrovascular Disease: A classification and outline of cerebrovascular disease. Part 2. Stroke 1975;6:564–616.

2 Ladurner G, Sager WD, Iliff LD, Lechner H: A correlation of clinical findings and CT in ischemic cerebrovascular disease. Eur Neurol 1979;18:281–288.

3 Dávalos A, Matías-Guiu J, Torrent O, Vilaseca J, Codina A: Computed tomography in reversible ischaemic attacks: clinical and prognostic correlations in a prospective study. J Neurol 1988;235:155–158.

4 Calandre L, Gomara S, Bermejo F, Millan JM, del Pozo G: Clinical-CT correlations in TIA, RIND, and strokes with minimum residuum. Stroke 1984;15:663–666.

5 Bogousslavsky J, Regli F: Cerebral infarct in apparent transient attack. Neurology 1985; 35:1501–1503.

6 Perrone P, Candelise L, Scotti G, De Grandi C, Scialfa G: CT evaluation in patients with transient ischemic attack. Correlation between clinical and angiographic findings. Eur Neurol 1979;18:217–221.

7 Fazekas F, Fazekas G, Schmidt R, Kapeller P, Offenbacher H: Magnetic resonance imaging correlates of transient cerebral ischemic attacks. Stroke 1996;27:607–611.

8 Kidwell CS, Alger JR, Di Salle F, Starkman S: Diffusion MRI in patients with transient ischemic attacks. Stroke 1999;30:1174–1180.

9 Adams HP Jr, Bendixen BH, Kappele LJ, Biller J, Love BB, Gordon DL, Marsh EE; the TOAST Investigators: Classification of subtype of acute ischemic stroke: definition for use in a multicenter clinical trial. Stroke 1993;24:35–41.

10 Rovira A, Rovira-Gols A, Pedraza S, Grivé E, Molina C, Alvarez-Sabín J: Diffusion-weighted MR imaging in the acute phase of transient ischemic attacks. AJNR Am J Neuroradiol 2002;23:77–83.

11 Crisostomo RA, García MM, Tong DC: Detection of diffusion-weighted MRI abnormalities in patients with transient ischemic attack. Correlation with clinical characteristics. Stroke 2003;34:932–937.

12 Inatomi Y, Kimura K, Yonehara T, Fujioka S, Uchino M: DWI abnormalities and clinical characteristics in TIA patients. Neurology 2004;62:376–380.

13 Takayama H, Mihara B, Kobayashi M, Hozumi A, Sadanaga H, Gomi S: Usefulness of diffusion-weighted MRI in the diagnosis of transient ischemic attacks (in Japanese). No To Shinkei 2000;52:919–923.

14 Engelter ST, Provenzale JM, Petrella JR, Alberts MJ: Diffusion MRI imaging and transient ischemic attacks. Stroke 1999;30:2762–2763.

15 Bisschops RHC, Kappelle LJ, Mali WPTM, van der Grond J: Hemodynamic and metabolic changes in transient ischemic attack patients. A magnetic resonance angiography and ^{1}H-magnetic resonance spectroscopy study performed within 3 days of onset of a transient ischemic attack. Stroke 2002;33: 110–115.

16 Nakamura T, Uchiyama S, Shibagaki Y, Iwata M: Abnormalities on diffusion-weighted magnetic resonance imaging in patients with transient ischemic attack. Clin Neurol 2003;43:122–125.

17 Li F, Silva MD, Sotak CH, Fisher M: Temporal evolution of ischemic injury evaluated with diffusion-, perfusion-, and T_2-weighted MRI. Neurology 2000;54:689–696.

18 Johnston SC, Gress DR, Browner WS, Sidney S: Short-term prognosis after emergency department diagnosis of TIA. JAMA 2000;284: 2901–2906.

Baseline NIH Stroke Scale Score predicting outcome in anterior and posterior circulation strokes

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ABSTRACT

Objective: The NIH Stroke Scale (NIHSS) may not appropriately assess the spectrum of posterior circulation (PC)–related neurologic deficits. We determined the cutoff baseline NIHSS score that predicts independent daily life activity during the chronic stage in anterior circulation (AC) vs PC ischemic strokes.

Methods: A total of 310 consecutive patients hospitalized within 3 days after the onset of an ischemic stroke were prospectively enrolled in the study. Patients on thrombolytic therapy were excluded. In all patients, infarcts and vascular lesions were identified primarily using magnetic resonance techniques. A favorable outcome was defined as a modified Rankin Scale score of ≤2 at 3 months poststroke.

Results: In 101 patients with PC stroke, the total baseline NIHSS score was lower ($p < 0.001$), and the subscores of ataxia ($p < 0.001$) and visual fields ($p = 0.043$) were higher than in 209 patients with AC stroke. Multivariate-adjusted OR for the favorable outcome in patients with PC vs AC stroke was 2.339 (95% CI 1.331–4.109, $p = 0.003$). A low baseline NIHSS score was independently predictive of a favorable outcome in both patients with PC (OR 1.547, 95% CI 1.232–1.941) and AC (1.279, 1.188–1.376) stroke. The optimal cutoff scores of the baseline NIHSS for the favorable outcome were ≤5 for patients with PC stroke (sensitivity, 84%; specificity, 81%) and ≤8 for patients with AC stroke (sensitivity, 80%; specificity, 82%).

Conclusions: The cutoff score of the baseline NIH Stroke Scale (NIHSS) for a favorable chronic outcome was relatively low in patients with PC stroke compared to patients with AC stroke. The NIHSS appears to have limitations with respect to its use when comparing the neurologic severity of PC and AC stroke. ***Neurology*® 2008;70:1-1**

GLOSSARY

AC = anterior circulation; **AUC** = area under the ROC curve; **mRS** = modified Rankin Scale; **NIHSS** = NIH Stroke Scale; **OCSP** = Oxfordshire Community Stroke Project; **PC** = posterior circulation; **ROC** = receiver operating characteristic; **rt-PA** = recombinant tissue plasminogen activator; **TOAST** = Trial of ORG 10172 in Acute Stroke Treatment.

The NIH Stroke Scale (NIHSS) is a neurologic severity scale that is valid, reliable, and reproducible[1-4]; it is commonly used in many clinical trials dealing with medical therapy for acute stroke.[5,6] Baseline NIHSS scores on admission are associated with chronic functional outcome,[6-8] hospital disposition after stroke,[9,10] infarct volume, and angiographic findings.[11-13]

In the Trial of ORG 10172 in Acute Stroke Treatment (TOAST), the baseline NIHSS score was lower in patients with posterior circulation (PC) stroke than in patients with anterior circulation (AC) stroke.[14] In a single-center study of Chinese patients with stroke, a severe baseline NIHSS score (≥9) was less frequent in patients with PC stroke than in patients with other stroke subtypes determined according to the Oxfordshire Community Stroke Project (OCSP) classification.[15] These results foretell that the long-term chronic outcome was also better in patients with PC stroke than in patients with AC

e-Pub ahead of print at www.neurology.org.
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Supported in part by Grants-in-Aid from the Ministry of Health, Labor and Welfare, Japan (H18-Junkanki-044).
Disclosure: The authors report no disclosures.

stroke. In fact, in the TOAST study, a favorable outcome at 3 months was more common in patients with PC stroke than in patients with AC stroke, although the outcome was no longer different after adjusting for confounders, including the baseline NIHSS score.[14] It has been reported that a favorable outcome was more common in patients with PC stroke (71%) than in patients with a partial (58%) or a total (3%) AC stroke.[16] These studies using quantitative scales have shown that, unlike what has previously been thought,[17] patients with PC stroke do not have a poorer prognosis than patients with AC stroke. However, these results highlight the important issue of whether the NIHSS is valid for comparing the neurologic severity of AC and PC stroke.

To resolve this issue, we assessed the NIHSS score on admission and the chronic outcome at 3 months, as well as the underlying clinical characteristics, of consecutive patients with stroke in a single stroke center. The aim of the present study was to compare how the baseline NIHSS score predicts chronic outcome in AC and PC ischemic stroke.

METHODS The study had a prospective observational cohort design. Patients who were admitted to our stroke center from December 2004 through December 2005 were enrolled. A total of 387 consecutive patients with acute ischemic stroke who were hospitalized within 3 days after symptom onset were registered. Of them, the following 77 patients were ineligible and were excluded from the study: 1) those receiving intra-arterial (3 patients) or IV (7 patients) thrombolytic therapy (all for the AC stroke); 2) those who were dependent with respect to their activities of daily living prior to the stroke (corresponding to a modified Rankin Scale [mRS] score of ≥ 3: 29 patients); 3) those with fresh ischemic lesions in both AC and PC territories (29 patients); 4) those with no relevant ischemic lesion on brain imaging (9 patients). The Regional Ethics and Hospital Management Committees approved the study. Written informed consent to participate in the study was obtained from the patient whenever possible; assent from a relative was obtained if patients could not consent themselves.

Baseline data were collected for all eligible patients, including gender, age, comorbidities, and neurologic deficits using the NIHSS on admission. The NIHSS subitems were also analyzed. The functional outcome was assessed at 3 months using the mRS determined by clinical examination (or by a mail-in survey for patients with too severe neurologic deficits to visit the clinic). A favorable outcome was defined as a mRS score of 0 to 2 (patients can look after their own affairs without assistance). The outcome corresponding to mRS score of 0 to 1 (patients can carry out all usual duties and activities) was also assessed. Death was coded as mRS 6. All patients had 12-lead electrocardiography, 72-hour electrocardiography monitoring, and transthoracic or transesophageal echocardiography. The location of infarcts was principally verified by diffusion-weighted MRI. In 11 patients for whom MRI was contraindicated, the fresh infarct was identified using repeated CT. In all patients, vascular lesions were verified by MRA (unless contraindicated) and carotid duplex sonography; conventional angiography was performed if needed. Stroke subtype was classified according to the TOAST categories.[18]

Statistical analysis was performed using the SPSS 11.0J statistical software package (SPSS Inc.). Between patients with AC and PC stroke, baseline clinical characteristics were compared using χ^2 tests and unpaired t tests, and NIHSS subscores were analyzed using the Mann-Whitney U test. OR for the favorable functional outcome in overall patients, corresponding to the mRS score of 0 to 2, according to the location of infarcts was determined using univariate or multivariate logistic regression analyses after adjustment for age, gender, and stroke subtypes (established clinical determinants of the stroke outcome). The baseline NIHSS score, another known determinant of the stroke outcome, was not used for the adjustment because we premised that the scale treats PC and AC strokes differently. To identify independent predictors for favorable outcome in patients with AC stroke and PC stroke separately, multivariate logistic regression analyses were performed using age, gender, stroke subtype, baseline NIHSS score, and the risk factors and comorbidities that showed the association with $p < 0.15$ in the univariate logistic regression analyses for patients with either AC or PC stroke. To obtain the baseline NIHSS score as the cutoff point for discriminating between patients with a favorable outcome and those without, we constructed receiver operating characteristic (ROC) curves and calculated the area under the ROC curve (AUC) with 95% CIs. $p < 0.05$ was considered significant.

RESULTS A total of 310 patients (202 men, 108 women; aged 71 ± 11 years) were enrolled in this study. A total of 209 patients had infarcts in the AC territory; 101 had infarcts in the PC territory.

Clinical characteristics of patients are presented in table 1 and figure 1. Patients with PC stroke were younger than patients with AC stroke ($p = 0.008$). There were no significant differences between the two groups in gender, comorbidities, or stroke subtypes. The baseline NIHSS score ($p < 0.001$) and the mRS at 3 months ($p = 0.001$) were lower in patients with PC stroke than in patients with AC stroke. Compared with patients with AC stroke, the unadjusted OR for the mRS score of 0 to 2 in patients with PC stroke was 2.313 (95% CI 1.371–3.901, $p = 0.002$), and the multivariate-adjusted OR was 2.339 (95% CI 1.331–4.109, $p = 0.003$).

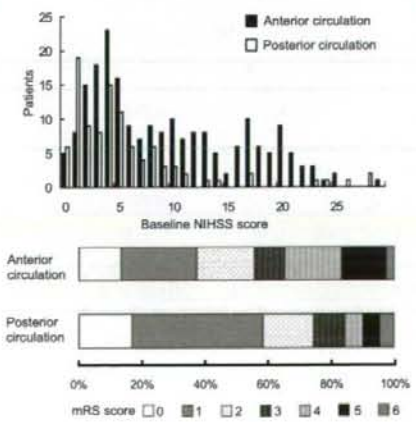
Table 1 Patient demographics, comorbidities, baseline NIH Stroke Scale (NIHSS), and chronic outcome

	Anterior circulation (n = 209)	Posterior circulation (n = 101)	p Value
Demographics			
Age, y	72 ± 10	68 ± 12	0.008
Gender, male, n (%)	135 (65)	67 (66)	0.800
Comorbidities, n (%)			
Previous stroke	59 (28)	31 (33)	0.403
Hypertension	152 (73)	71 (70)	0.687
Diabetes mellitus	65 (31)	39 (39)	0.201
Hyperlipidemia	83 (40)	36 (36)	0.534
Ischemic heart disease	39 (19)	16 (16)	0.635
Valvular heart disease	15 (7)	9 (9)	0.652
Atrial fibrillation	77 (37)	33 (33)	0.527
Peripheral artery disease	15 (7)	5 (5)	0.623
Stroke subtype, n (%)			0.160
Small-vessel	35 (17)	15 (15)	
Cardioembolism	92 (44)	34 (34)	
Large-artery	33 (16)	25 (25)	
Other/undetermined	49 (23)	27 (27)	
Baseline NIHSS score, median (interquartile range)	8 (4-15)	4 (2-7)	<0.001
mRS score at 3 months, median (interquartile range)	2 (1-4)	1 (1-3)	0.001

mRS = modified Rankin Scale.

Table 2 shows the distribution of individual baseline NIHSS subscores. Items of the NIHSS score for ataxia and visual fields were higher in patients with PC stroke than in patients with AC stroke. Most of the other NIHSS item scores, such as level of consciousness, gaze, facial palsy, motor arm, motor leg, language, and extinction/inattention, were higher in patients with AC stroke than in patients with PC stroke.

Figure 1 Distribution of the baseline NIH Stroke Scale (NIHSS) score and modified Rankin Scale (mRS) score at 3 months



Univariate and multivariate analyses done to determine predictors of a favorable chronic outcome are shown in table 3. A lower baseline NIHSS score was independently predictive of a favorable outcome for patients with AC (OR 1.279, 95% CI 1.188–1.376) and PC (OR 1.547, 95% CI 1.232–1.941) stroke. In addition, ischemic heart disease was independently predictive of an unfavorable outcome (mRS 3–6) in patients with PC stroke.

The AUC of the ROC curve for predicting a favorable outcome, corresponding to mRS of 0 to 2, in patients with PC stroke (0.867, 95% CI 0.783–0.951) was similar to that in patients with AC stroke (0.868, 95% CI 0.818–0.917, figure 2). For patients with PC stroke, the optimal cutoff score of the baseline NIHSS was ≤5 with a sensitivity of 84% (95% CI 74–92%), specificity of 81% (61–93%), positive predictive value of 93% (84–98%), and negative predictive value of 64% (45–80%). For patients with AC stroke, the optimal cutoff score was ≤8 with a sensitivity of 80% (95% CI 72–87%), specificity of 82% (72–89%), positive predictive value of 85% (76–91%), and negative predictive value of 77% (67–85%). For patients with AC stroke, the sensitivity of a baseline NIHSS score ≤5 to predict a favorable outcome was 65% (95% CI 55–73%), and the specificity was 89% (81–95%). For patients with PC stroke, the specificity of a baseline NIHSS ≤8 to predict a favorable outcome was 46% (95% CI 27–67%), and the sensitivity was 93% (85–98%).

The analysis was also performed for predicting the outcome, corresponding to mRS of 0 to 1. The AUC of the ROC curve in patients with PC stroke was 0.896 (95% CI 0.834–0.958) and that in patients with AC stroke was 0.801 (95% CI 0.741–0.861). For patients with PC stroke, the optimal cutoff score of the baseline NIHSS was ≤5 with a sensitivity of 93% (95% CI 84–98%) and specificity of 69% (53–82%). For patients with AC stroke, the optimal cutoff score was ≤8 with a sensitivity of 85% (95% CI 75–92%) and specificity of 66% (95% CI 58–74%).

DISCUSSION In this study, we assessed the relationship between baseline neurologic severities evaluated using the NIHSS and the chronic outcome of patients with ischemic strokes involving different arterial territories, determined primarily

Table 2 Baseline NIH Stroke Scale subscores

	Anterior circulation (n = 198)					Posterior circulation (n = 96)					
Items	0	1	2	3	4	0	1	2	3	4	p Value
#1A Level of consciousness	115	68	15			78	16	1	1		<0.001
#1B Questions	126	20	52			86	5	5			<0.001
#1C Commands	155	16	27			93	1	2			<0.001
#2 Gaze	141	46	11			85	9	2			0.001
#3 Visual fields	177	17	4			78	11	5	2		0.043*
#4 Facial palsy	61	87	50			56	34	5	1		<0.001
#5 Motor arm											
a. Left	124	28	12	12	22	73	10	9	3	1	0.010
b. Right	110	40	16	16	16	67	17	7	3	2	0.008
#6 Motor leg											
a. Left	120	30	18	16	14	71	12	10	1	2	0.011
b. Right	110	33	24	12	19	70	11	11	1	3	0.002
#7 Ataxia	186	8	4			67	19	10			<0.001*
#8 Sensory	98	79	20	1		55	37	4			0.111
#9 Language	137	18	19	24		88	5	1	2		<0.001
#10 Dysarthria	70	99	29			41	45	10			0.168
#11 Extinction/inattention	140	31	27			92	4				<0.001

Number indicates patient number. Eleven patients with anterior circulation stroke and five patients with posterior circulation stroke were excluded because of missing data.
*The posterior circulation group's scores are higher than those of the anterior circulation group.

using diffusion-weighted MRI. The major findings of this study were that optimal cutoff NIHSS scores to predict a favorable chronic outcome differed in patients with AC and PC stroke, and patients with PC stroke had a high probability of an unfavorable outcome at 3 months with relatively low NIHSS scores.

Although the NIHSS is the most widely used scoring system in patients with stroke and is highly predictive of chronic outcome, it has a potential weakness with respect to uneven scoring of lesion-specific neurologic deficits. For example, a right hemispheric stroke receives a low NIHSS score compared with the same-sized left hemispheric stroke, partly because the NIHSS awards seven points for tests directly related to language function and only two points for neglect.[11,19] There appear to be similar concerns when the NIHSS is used to compare patients with AC and PC stroke. The scale is highly weighted toward AC deficits, including cortical signs and motor function, while PC deficits, including cranial nerve signs and ataxia, receive fewer points[4,14]; ataxia is frequently excluded from scoring due to the coexistence of motor deficits. Thus, NIHSS may not appropriately evaluate the spectrum of PC-related signs. This weakness seems to be an important cause of the difference between AC and PC stroke in the NIHSS cutoff scores for predicting chronic outcome. Even the mRS has a weakness in that it is highly focused on the ability to walk and perform the usual activities of daily living.[4,20]

At present, thrombolysis using IV recombinant tissue plasminogen activator (rt-PA) is the most effective therapy for acute ischemic stroke. In several key thrombolysis studies, the NIHSS has always been used to assess baseline patient severity and sometimes to assess final outcome.[5,21-23] In a series of infarcts presenting within 3 hours, the presence of mild symptoms was the leading reason why patients did not receive thrombolysis.[24] Some trials excluded patients with baseline NIHSS scores of ≤4, as they were considered to have had a mild stroke[22,23]; clinically, such patients may be considered ineligible for IV rt-PA. However, the NIHSS cutoff score predicting a favorable outcome for patients with PC stroke (≤5) is close to 4; thus, one should not simply refrain from thrombolysis in patients with PC stroke with a relatively low NIHSS score, but one should consider thrombolysis if the PC-specific deficits that were underestimated by NIHSS warrant rt-PA. A recent study warned that a substantial

Table 3 Univariate and multivariate logistic regression analysis of the probability of a favorable outcome (modified Rankin Scale 0–2) at 3 months

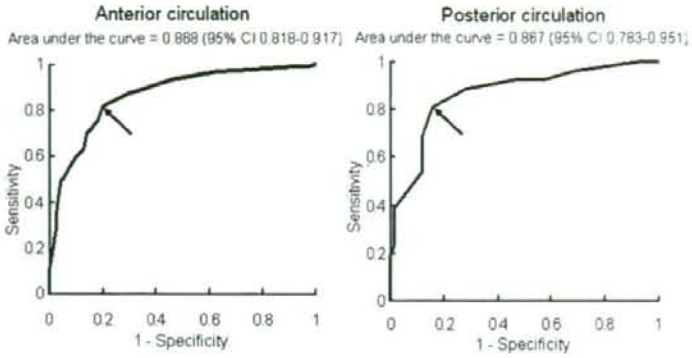
	Univariate analysis			Multivariate analysis		
Variables	Favorable	Unfavorable	p Value	OR	95% CI	p Value
Anterior circulation (n = 209)						
Age	71 ± 10	73 ± 10	0.976	0.998	0.959–1.038	0.910
Gender, male	82 (71%)	53 (57%)	0.549	1.276	0.584–2.788	0.542
Previous stroke	37 (32%)	22 (24%)	0.190			
Hypertension	86 (74%)	66 (71%)	0.609			
Diabetes mellitus	33 (28%)	32 (34%)	0.355			
Hyperlipidemia	43 (37%)	40 (43%)	0.383			
Ischemic heart disease	19 (16%)	20 (22%)	0.346	0.744	0.301–1.839	0.522
Valvular heart disease	7 (6%)	8 (9%)	0.477			
Atrial fibrillation	35 (30%)	42 (45%)	0.026	1.282	0.497–3.308	0.607
Peripheral artery disease	9 (8%)	6 (7%)	0.716			
Stroke subtype						
Small-vessel	30 (26%)	5 (5%)	<0.001	2.034	0.541–7.648	0.293
Cardioembolism	34 (29%)	58 (62%)	<0.001	0.862	0.257–2.898	0.811
Large-artery	17 (15%)	16 (17%)	0.616			
Other/undetermined	35 (30%)	14 (15%)	0.012	2.547	0.788–8.229	0.118
Baseline NIHSS score, median (interquartile range)	4 (3–7)	15 (10–19)	<0.001	1.279	1.188–1.376	<0.001
Posterior circulation (n = 101)						
Age	67 ± 12	72 ± 11	0.124	1.023	0.963–1.087	0.456
Gender, male	53 (71%)	14 (54%)	0.121	2.745	0.686–10.980	0.153
Previous stroke	22 (32%)	9 (36%)	0.708			
Hypertension	53 (71%)	18 (69%)	0.890			
Diabetes mellitus	26 (35%)	13 (50%)	0.169			
Hyperlipidemia	28 (37%)	8 (31%)	0.548			
Ischemic heart disease	9 (12%)	7 (27%)	0.080	0.103	0.020–0.544	0.007
Valvular heart disease	6 (8%)	3 (12%)	0.587			
Atrial fibrillation	23 (31%)	10 (39%)	0.466	0.739	0.130–4.189	0.733
Peripheral artery disease	4 (5%)	1 (4%)	0.764			
Stroke subtype						
Small-vessel	13 (17%)	2 (8%)	0.247	0.546	0.059–5.052	0.594
Cardioembolism	22 (29%)	12 (46%)	0.121	0.364	0.057–2.318	0.285
Large-artery	17 (23%)	8 (31%)	0.411			
Other/undetermined	23 (31%)	4 (15%)	0.138	1.518	0.201–11.489	0.686
Baseline NIHSS score, median (interquartile range)	3 (1–5)	8 (6–14)	<0.001	1.547	1.232–1.941	<0.001

NIHSS = NIH Stroke Scale.

minority of patients who were deemed to have symptoms that were too mild to warrant IV rt-PA were unable to be discharged home.[25]

Since MRI and MRA including diffusion-weighted imaging were performed in this study for all consecutive patients with stroke unless contraindicated, the classification of stroke into AC and PC groups was highly reliable. This study had a few limitations. Since our series is from a single, highly specialized medical center that deals with cardiovascular diseases and emergent cases, the percentage of cardioembolism was high compared to a typical Japanese epidemiologic study.[26] Furthermore, the small study population might have introduced statistical error. Because this is a development model and has not been validated, our findings should only be considered hypothesis creating,

Figure 2 Receiver operating characteristic (ROC) curves to show optimal cutoff point of the baseline NIH Stroke Scale (NIHSS) scores of the patients with anterior circulation (AC) and posterior circulation (PC) stroke to predict a favorable outcome (modified Rankin Scale of 0–2)



Arrows indicate optimal cutoff points.

and applicability and generalizability of our models have not yet been evaluated.

ACKNOWLEDGMENT

The authors thank Akiko Kada, MPH, for advice on the statistical analyses.

Received June 19, 2007. Accepted in final form December 7, 2007.

REFERENCES

1. Brott T, Adams Jr. HP, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864–870.
2. Goldstein LB, Bertels C, Davis JN. Interrater reliability of the NIH Stroke Scale. Arch Neurol 1989;46:660–662.
3. Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group.Stroke1994;25:2220–2226.
4. Kasner SE. Clinical interpretation and use of stroke scales. Lancet Neurol 2006;5:603–612.
5. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995; 333:1581–1587.
6. Adams Jr. HP, Davis PH, Leira EC, et al. Baseline NIH Stroke Scale score strongly predicts outcome after stroke: a report of the Trial of Org 10172 in Acute Stroke Treatment (TOAST). Neurology 1999;53:126–131.
7. Frankel MR, Morgenstern LB, Kwiatkowski T, et al. Predicting prognosis after stroke: a placebo group analysis from the National Institute of Neurological Disorders and Stroke rt-PA Stroke Trial. Neurology 2000;55: 952–959.
8. Weimar C, Konig IR, Kraywinkel K, Ziegler A, Diener HC. Age and National Institutes of Health Stroke Scale Score within 6 hours after onset are accurate predictors of outcome after cerebral ischemia: development and external validation of prognostic models. Stroke 2004; 35:158–162.
9. Schlegel D, Kolb SJ, Luciano JM, et al. Utility of the NIH Stroke Scale as a predictor of hospital disposition. Stroke 2003;34:134–137.
10. Schlegel DJ, Tanne D, Demchuk AM, Levine SR, Kasner SE. Prediction of hospital disposition after thrombolysis for acute ischemic stroke using the National Institutes of Health Stroke Scale. Arch Neurol 2004;61: 1061–1064.
11. Fink JN, Selim MH, Kumar S, et al. Is the association of National Institutes of Health Stroke Scale scores and acute magnetic resonance imaging stroke volume equal for patients with right- and left-hemisphere ischemic stroke? Stroke 2002;33:954–958.
12. Nakajima M, Kimura K, Ogata T, Takada T, Uchino M, Minematsu K. Relationships between angiographic findings and National Institutes of Health stroke scale score in cases of hyperacute carotid ischemic stroke. AJNR Am J Neuroradiol 2004;25:238–241.
13. Fischer U, Arnold M, Nedeltchev K, et al. NIHSS score and arteriographic findings in acute ischemic stroke. Stroke 2005;36:2121–2125.
14. Libman RB, Kwiatkowski TG, Hansen MD, Clarke WR, Woolson RF, Adams HP. Differences between anterior and posterior circulation stroke in TOAST. Cerebrovasc Dis 2001;11:311–316.
15. Li H, Wong KS, Kay R. Relationship between the Oxfordshire Community Stroke Project classification and vascular abnormalities in patients with predominantly intracranial atherosclerosis. J Neurol Sci 2003;207:65–69.
16. Pinto AN, Melo TP, Lourenco ME, et al. Can a clinical classification of stroke predict complications and treatments during hospitalization? Cerebrovasc Dis 1998;8: 204–209.
17. Jones Jr. HR, Millikan CH, Sandok BA. Temporal profile (clinical course) of acute vertebrobasilar system cerebral infarction. Stroke 1980;11:173–177.
18. Adams Jr. HP, Bendixen BH Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Stroke 1993; 24:35–41.
19. Woo D, Broderick JP, Kothari RU, et al. Does the National Institutes of Health Stroke Scale favor left hemisphere strokes? NINDS t-PA Stroke Study Group. Stroke 1999;30:2355–2359.
20. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19: 604–607.
21. Clark WM, Wissman S, Albers GW, Jhamandas JH, Madden KP, Hamilton S. Recombinant tissue-type plasminogen activator (alteplase) for ischemic stroke 3 to 5 hours after symptom onset: the ATLANTIS Study: a randomized controlled trial: Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke. JAMA 1999;282:2019–2026.
22. 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 2005;36: 66–73.
23. Yamaguchi T, Mori E, Minematsu K, et al. Alteplase at 0.6 mg/kg for acute ischemic stroke within 3 hours of

onset: Japan Alteplase Clinical Trial (J-ACT). Stroke 2006;37:1810–1815.
24. Barber PA, Zhang J, Demchuk AM, Hill MD, Buchan AM. Why are stroke patients excluded from TPA therapy? An analysis of patient eligibility. Neurology 2001; 56:1015–1020.
25. Smith EE, Abdullah AR, Petkovska I, Rosenthal E, Koroshetz WJ, Schwamm LH. Poor outcomes in patients who do not receive intravenous tissue plasminogen activator because of mild or improving ischemic stroke. Stroke 2005;36:2497–2499.
26. Kimura K, Kazui S, Minematsu K, Yamaguchi T. Analysis of 16,922 patients with acute ischemic stroke and transient ischemic attack in Japan: a hospital-based prospective registration study. Cerebrovasc Dis 2004; 18:47–56.

Journal of Cerebral Blood Flow & Metabolism (2008) 28, 445–449

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Brief Communication

Circulating CD34-positive cells provide a marker of vascular risk associated with cognitive impairment

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Maintenance of uninterrupted cerebral circulation is critical for neural homeostasis. The level of circulating CD34-positive (CD34$^+$) cells has been suggested as an index of cerebrovascular health, although its relationship with cognitive function has not yet been defined. In a group of individuals with cognitive impairment, the level of circulating CD34$^+$ cells was quantified and correlated with clinical diagnoses. Compared with normal subjects, a significant decrease in circulating CD34$^+$ cells was observed in patients with vascular-type cognitive impairment, although no significant change was observed in patients with Alzheimer's-type cognitive impairment who had no evidence of cerebral ischemia. The level of cognitive impairment was inversely correlated with numbers of circulating CD34$^+$ cells in patients with vascular-type cognitive impairment, but not Alzheimer's type. We propose that the level of circulating CD34$^+$ cells provides a marker of vascular risk associated with cognitive impairment, and that differences in the pathobiology of Alzheimer's- and vascular-type cognitive impairment may be mirrored in levels of circulating CD34$^+$ cells in these patient populations.

Journal of Cerebral Blood Flow & Metabolism (2008) **28,** 445–449; doi:10.1038/sj.jcbfm.9600541; published online 8 August 2007

Keywords: antigens; CD34; cerebral circulation; cognitive impairment

Introduction

Maintaining integrity of the cerebral circulation has a critical role in neural homeostasis. Although analysis of risk factors for cerebrovascular disease has certainly provided insights into mechanisms of vascular disease, it is still difficult to predict accurately the contribution of vascular dysfunction in the long-term outcome of acute vascular insufficiency or in chronic neurodegenerative disorders. For example, in Alzheimer's disease (Casserly and Topol, 2004; Vagnucci and Li, 2003), assessment of a possible vascular component in the pathogenesis of neuronal degeneration is often ambiguous during a patient's lifetime.

Repair of the cerebral microcirculation has traditionally been assigned to ongoing replacement of damaged cerebral endothelium from outgrowth of preexisting vasculature. However, recent studies have identified circulating bone marrow-derived immature cells, including CD34-positive (CD34$^+$) cells, as contributors in maintenance of the vasculature; they have the potential to serve as a pool of endothelial progenitor cells (Asahara *et al*, 1997) and as a source of growth/angiogenesis factors (Majka *et al*, 2001). In a previous study, we have shown that circulating CD34$^+$ cells provide an index of cerebrovascular function (Taguchi *et al*, 2004*a*). We have also found that in a model of experimental cerebral ischemia, intravenous administration of CD34$^+$ cells improved neurologic function, at least in part, by restoring cerebral microcirculation in the ischemic area (Taguchi *et al*, 2004*b*).

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This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare.
Received 7 June 2007; revised 1 July 2007; accepted 2 July 2007; published online 8 August 2007

These results lead us to propose that circulating immature vascular progenitor cells contribute to neural homeostasis, at least in part, through their role in maintaining cerebral microvascular function. Using a recently developed method that allows precise measurement of the CD34+ cell population in peripheral blood (Kikuchi-Taura *et al*, 2006), we have evaluated the level of circulating CD34+ cells in patients with impaired neurologic function of diverse etiologies. Our goal has been to determine if there is relationship between levels of CD34+ cells, impaired neural function, and vascular integrity.

Materials and methods

This study was approved by Institutional Review Boards of the respective institutions (National Cardiovascular Center, Hyogo College of Medicine, Hoshigaoka Koseinenkin Hospital, and Osaka Minami National Medical Center). All subjects provided informed consent. Individuals with Mini Mental State Examination Score (MMSE) <24 and Clinical Dementia Rating (CDR) ≥ 0.5 were enrolled in this study and defined as having impaired cognitive function. In the view of history, evaluation of symptoms, and results of brain imaging studies (magnetic resonance imaging and single photon-computed tomography), patients with cognitive impairment were divided into two groups by neurologists blinded to the experimental protocol: vascular-type cognitive impairment or Alzheimer's-type cognitive impairment, according to the criteria of *Diagnostic and Statistical Manual of Mental Disorders* (4th ed, DSM-4) (American Psychiatric Association, 1994). To exclude the contribution of vascular element in patients with Alzheimer's-type cognitive impairment, patients' coexistent Alzheimer's-type cognitive impairment and cerebral infarction, observed by magnetic resonance imaging, were excluded from this study. In addition, patients with cognitive impairment diagnosed as neither of the Alzheimer's type nor vascular type were excluded. A total of 95 individuals, including 32 age-matched control subjects with no history of vascular disease, no neuronal deficiency, and no cognitive impairment, were enrolled. In addition, individuals excluded from the study included: premenopausal women, patients who experienced a vascular event within 30 days of measurements, history of cerebral hemorrhage, and evidence of infection or malignant disease. Using a modification of the International Society of Hematotherapy and Graft Engineering (ISHAGE) Guidelines (Sutherland *et al*, 1996), the number of circulating CD34+ cells was quantified as described (Kikuchi-Taura *et al*, 2006). In brief, blood samples were incubated with phycoerythrin-labeled anti-CD34 antibody, fluorescein isothiocyanate-labeled anti-CD45 antibody, 7-aminoactinomycin-D, and internal control (all of these reagents are from the Stem-Kit, Beckman Coulter, Marseille, France). 7-Aminoactinomycin-D-positive dead cells and CD45-negative cells were excluded, and the number of cells forming a cluster with characteristic CD34+ cells (i.e., low side scatter and low-to-intermediate CD45 staining) was counted. The absolute number of CD34+ cells was calculated using the internal control. In this study, we used a single measurement at the time of entry into the study, on the basis of our previous observation that the level of circulating CD34+ cells is relatively stable (Taguchi *et al*, 2004*a*). For statistical analysis, JMP version 5.1J (SAS Institute Inc, Co, NC, USA) was used. Individual comparisons were performed using a two-tailed, unpaired Students' *t*-test. Statistical comparisons among groups were determined using analysis of variance. Mean $\pm$ s.e. is shown.

Results

Baseline characteristics of the groups are shown in Table 1. In univariate analysis of control subjects, each cerebrovascular risk factor and other treatment showed no significant difference with the number of circulating CD34+ cells (data not shown).

To investigate a possible relationship between circulating CD34+ cells and cognition, the level of circulating CD34+ cells was compared among these groups. Representative fluorescence-activated cell sorting images are shown in Figure 1A (vascular-type) and 1B (Alzheimer's-type). Analysis of variance revealed a significant decrease of CD34+ cells in patients with vascular-type cognitive impairment compared with Alzheimer's-type cognitive impairment ($P<0.001$) and normal subjects ($P<0.001$, Figure 1C).

To investigate further a possible association of circulating CD34+ cells with cognitive impairment, patients with vascular-type impaired cognition were divided into two groups according to their CDR (mild: CDR = 0.5, $n=22$, mean age = 75.2 ± 1.6 years; moderate-severe: CDR ≥ 1, $n=18$, mean age = 75.3 ± 1.5 years) or MMSE (mild: MMSE ≥ 20, $n=25$, mean age = 74.2 ± 1.4 years; moderate-severe: MMSE <20, $n=15$, mean age = 77.1 ± 1.5 years). The results showed a significant decrease in the level of circulating CD34+ cells in moderate-severe group, based on stratification by either CDR (Figure 1D, $P=0.01$) or MMSE (Figure 1E, $P=0.03$) in patients with vascular-type cognitive impairment. Similar analysis was applied to patients with Alzheimer's-type impaired cognition. They were divided into two groups according to CDR (mild: $n=8$, mean age = 73.0 ± 4.7 years; moderate-severe: $n=15$, mean age = 77.5 ± 1.9 years) or MMSE (mild: $n=12$, mean age = 74.1 ± 3.0 years; moderate-severe: $n=11$, mean age = 77.8 ± 2.9 years). However, in contrast to patients with vascular-type impaired cognition, there was no significant difference observed in patients with Alzheimer's-type cognitive impaired, based on CDR (Figure 1F, $P=0.86$) or MMSE (Figure 1G, $P=0.60$).

Discussion

Our results are consistent with a contribution of circulating CD34+ cells in support of cognitive function, presumably through their positive homeostatic influence on the cerebral circulation in

Table 1 Baseline characteristics

	Total	Cognitive impairment		Control	P-value for trend
		Vascular-type	Alzheimer's-type		
n	95	40	23	32	
Age, years	74.9±0.6	75.3±1.1	75.9±2.1	74.2±0.7	0.53
Male gender, *n* (%)	57 (60)	27 (68)	12 (52)	18 (56)	0.46
Risk factor, n (%)					
Hypertension	41 (43)	21 (53)	9 (39)	11 (34)	0.28
Hyperlipidemia	29 (31)	14 (35)	5 (22)	10 (31)	0.53
Diabetes mellitus	9 (9)	5 (13)	1 (4)	3 (9)	0.57
Smoking	20 (21)	10 (25)	6(26)	4 (13)	0.34
Treatment, n (%)					
Ca-channel blocker	30 (32)	15 (38)	6 (26)	9 (28)	0.56
β-Blocker	2 (2)	1 (3)	0 (0)	1 (3)	0.71
ACE inhibitor	4 (4)	3 (8)	1 (4)	0 (0)	0.29
ARB	8 (8)	3 (8)	3 (13)	2 (6)	0.65
Diuretics	6 (6)	2 (5)	1 (4)	3(9)	0.68
Statin	29 (31)	14 (35)	5 (22)	10 (31)	0.54
Aspirin	28 (29)	23 (58)	1 (4)	4 (13)	<0.01
Ticlopidine	11(12)	9 (23)	0 (0)	2 (6)	0.01

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

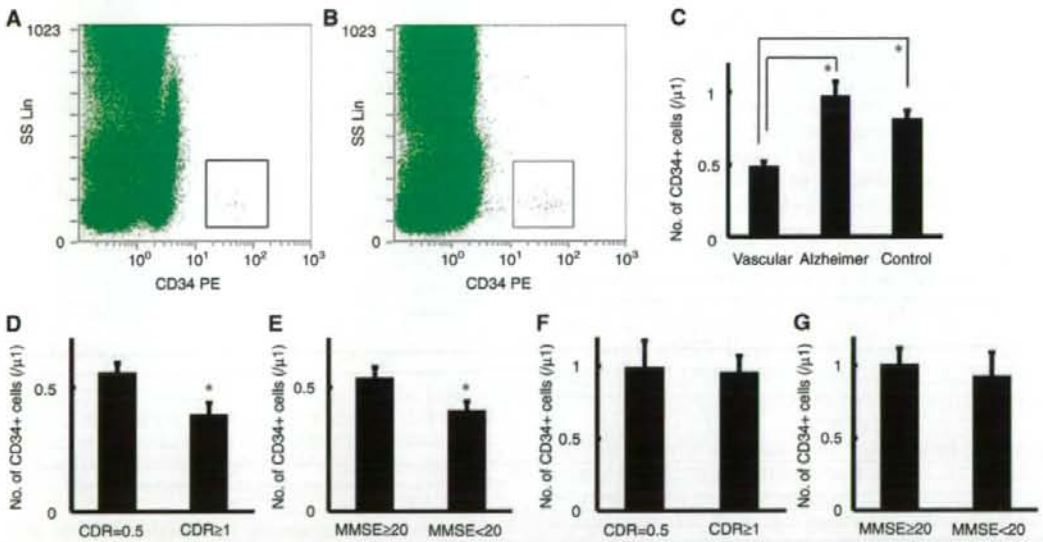

Figure 1 Levels of circulating CD34+ cells and cognitive impairment. (**A** and **B**) After exclusion of 7-AAD-positive dead cells and CD45-negative cells (non-leukocyte), CD34+ cells cluster at low side scatter were clearly observed (**A**, vascular-type; **B**, Alzheimer's-type). (**C**) Analysis of variance revealed a significant decrease in circulating CD34+ cells in patients with vascular-type cognitive impairment compared with normal subjects and individuals with Alzheimer's-type cognitive impairment. In contrast, no significant change in circulating CD34+ cells was observed in patients with Alzheimer's-type cognitive impairment compared with control subjects. (**D** and **E**) In the group of patients with vascular-type cognitive impairment, the level of circulating CD34+ cells was significantly reduced in patients with more severe cognitive impairment compared with the more mildly affected group (**D**, CDR; **E**, MMSE). (**F** and **G**) In contrast, no significant difference was observed in patients with Alzheimer's-type cognitive impairment based on assessment of cognition (**F**, CDR; **G**, MMSE). SS Lin, side-scatter linear scale. $^*P < 0.05$.

settings of ischemic stress. Further, these observations suggest a basic difference between the pathobiology of dementia in Alzheimer's disease (without associated cerebral ischemia) and declining cognitive function in patients with ischemic cerebrovascular disorders.

Late onset, sporadic Alzheimer's disease is a heterogeneous disorder (Casserly and Topol, 2004) and the contribution of a vascular factor is still controversial. In contrast to vascular-type cognitive impairment, no significant change (at most, a mild increase) in the level of circulating CD34+ cells was observed in patients with Alzheimer's-type cognitive impairment who had no cerebral ischemia. Consistent with a CD34+ cell-independent mechanism of cognitive decline in Alzheimer's-type impaired cognition, there was no correlation between circulating CD34+ cells and the level of CDR or MMSE. These results suggest that the level of CD34+ cells in the peripheral circulation might provide a useful means of separating dementia with a vascular etiology from dementia associated with nonvascular causes. This is not inconsistent with a previous report indicating decreased levels of CD34+ cells in patients with early Alzheimer's disease that did not exclude patients with coexisting cerebral ischemia (Maler *et al*, 2006). Our findings could have implications for treatment, especially as more modalities become available for patients with declining cognitive function.

The level of circulating endothelial progenitor cells, identified based on positivity for CD34 and kinase insert domain receptor (CD34+/KDR+ cells), has been correlated with cardiovascular risk factors (Vasa *et al*, 2001) and cardiovascular outcomes (Schmidt-Lucke *et al*, 2005; Werner *et al*, 2005). However, large variations in the levels of CD34+/KDR+ cells in the latter reports (by ~100-fold between reports; Fadini *et al*, 2006; Werner *et al*, 2005) indicate the need to standardize this measurement. In contrast, in our study, although there was no strong correlation between levels of CD34+ cells and established cardiovascular risk factors and other treatments, probably because of the heterogeneity of our control subjects, the results indicate a close relationship between the overall CD34+ pool and the cognitive impairment with cerebral ischemia. Previous reports have indicated a positive correlation between mobilization of CD34+ cells and improved functional outcome in stroke patients (Dunac *et al*, 2007). Accelerated functional recovery after experimental stroke, because of administration of CD34+ cells (Shyu *et al*, 2006; Taguchi *et al*, 2004*b*), suggests the possible contribution of CD34+ cells in maintenance of brain function during cerebral circulation. Our method for quantification of CD34+ cells is simple, reproducible (Kikuchi-Taura *et al*, 2006), and suitable for screening a broad group of patients at risk for cerebrovascular disorders.

In conclusion, our results indicate that the level of circulating CD34+ cells provides a marker of vascular risk associated with cognitive impairment. Furthermore, differences in the pathobiology of Alzheimer's- and vascular-type cognitive impairment may be mirrored in levels of circulating CD34+ cells in these patient populations.

Acknowledgements

We thank Y Kasahara, K Obata, and Y Okinaka for technical assistance.

Conflict of interest

The authors state no conflict of interest.

References

American Psychiatric Association (1994) *Diagnostic and statistical manual of mental disorders.* 4th ed Washington, DC: American Psychiatric Association

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275:964–7

Casserly I, Topol E (2004) Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins. *Lancet* 363:1139–46

Dunac A, Frelin C, Popolo-Blondeau M, Chatel M, Mahagne MH, Philip PJ (2007) Neurological and functional recovery in human stroke are associated with peripheral blood CD34+ cell mobilization. *J Neurol* 254:327–32

Fadini GP, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A, de Kreutzenberg SV (2006) Peripheral blood CD34+KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population. *Stroke* 37:2277–82

Kikuchi-Taura A, Soma T, Matsuyama T, Stern DM, Taguchi A (2006) A new protocol for quantifying CD34+ cells in peripheral blood of patients with cardiovascular disease. *Texas Heart Inst J* 33:427–9

Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z, Kowalska MA, Gewirtz AM, Emerson SG, Ratajczak MZ (2001) Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood* 97: 3075–85

Maler JM, Spitzer P, Lewczuk P, Kornhuber J, Herrmann M, Wiltfang J (2006) Decreased circulating CD34+ stem cells in early Alzheimer's disease: evidence for a deficient hematopoietic brain support? *Mol Psychiatry* 11:1113–5

Schmidt-Lucke C, Rossig L, Fichtlscherer S, Vasa M, Britten M, Kamper U, Dimmeler S, Zeiher AM (2005) Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 111:2981–7

Shyu WC, Lin SZ, Chiang MF, Su CY, Li H (2006) Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. *J Neurosci* 26:3444–53

Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I (1996) The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J Hematother* 5:213–26

Taguchi A, Matsuyama T, Moriwaki H, Hayashi T, Hayashida K, Nagatsuka K, Todo K, Mori K, Stern DM, Soma T, Naritomi H (2004*a*) Circulating CD34-positive cells provide an index of cerebrovascular function. *Circulation* 109:2972–5

Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T (2004*b*) Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest* 114: 330–8

Vagnucci Jr AH, Li WW (2003) Alzheimer's disease and angiogenesis. *Lancet* 361:605–8

Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S (2001) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 89:E1–7

Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G (2005) Circulating endothelial progenitor cells and cardiovascular outcomes. *New Engl J Med* 353:999–1007

Journal of Cerebral Blood Flow & Metabolism (2008) 28, 1086–1089

www.jcbfm.com

Brief Communication

Increase in circulating CD34-positive cells in patients with angiographic evidence of moyamoya-like vessels

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Increasing evidence points to a role for circulating endothelial progenitor cells, including populations of CD34-positive (CD34$^+$) cells, in maintenance of cerebral blood flow. In this study, we investigated the link between the level of circulating CD34$^+$ cells and neovascularization at ischemic brain. Compared with control subjects, a remarkable increase of circulating CD34$^+$ cells was observed in patients with angiographic moyamoya vessels, although no significant change was observed in patients with major cerebral artery occlusion (or severe stenosis) but without moyamoya vessels. Our results suggest that the increased level of CD34$^+$ cells associated with ischemic stress is correlated with neovascularization at human ischemic brain.
Journal of Cerebral Blood Flow & Metabolism (2008) **28,** 1086–1089; doi:10.1038/jcbfm.2008.1; published online 30 January 2008

Keywords: antigens; CD34; moyamoya vessel; neovascularization

Introduction

Increasing evidence points to a role for bone marrow-derived immature cells, such as endothelial progenitor cells, in maintenance of vascular homeostasis and repair. CD34-positive (CD34$^+$) cells comprise a population enriched for endothelial progenitor cells whose contribution to neovasculature includes both direct participation in forming the neovessel and regulatory roles as sources of growth/angiogenesis factors (Majka *et al,* 2001). Previously, we have shown accelerated neovascularization after administration of CD34$^+$ cells in an experimental model of stroke (Taguchi *et al,* 2004*b*) and induced by autologous bone marrow mononuclear cells (rich cell fraction of CD34$^+$ cells) transplanted locally into patients with limb ischemia (Taguchi *et al,* 2003). In addition, we have observed a positive correlation between the level of circulating CD34$^+$ cells and regional blood flow (Taguchi *et al,* 2004*a*), and cognitive function (Taguchi *et al,* 2007) in patients with chronic cerebral ischemia.

In this study, we have evaluated the level of circulating CD34$^+$ cells in patients with unusually accelerated neovascularization induced by progressive occlusion (or severe stenosis) of the supraclinoid portion of the internal carotid artery, the proximal region of the anterior, and/or middle cerebral artery characterized angiographically by the presence of moyamoya-like vessels (Natori *et al,* 1997) that supply ischemic brain as collaterals. We have investigated the hypothesis that circulating bone marrow-derived immature cells might be associated with neovascularization at ischemic sites in the human brain.

Patients and methods

The institutional review board of the National Cardiovascular Center approved this study. All subjects provided

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This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare (H19-Choujyu-029).
Received 29 October 2007; revised 19 December 2007; accepted 26 December 2007; published online 30 January 2008

informed consent. A total of 50 individuals, including 24 patients with occlusion or severe stenosis (>90%) at the C1 portion of the internal carotid artery or the M1 portion of the middle cerebral artery, and 26 age-matched healthy volunteers with cardiovascular risk factors, but without history of vascular disease, were enrolled. The diagnosis of cerebral artery occlusion or stenosis was made angiographically and four patients were found to have classical angiographic evidence of moyamoya-like vessels, including one with right C1 occlusion, one with right M1 occlusion, and two with bilateral C1 severe stenosis. All patients with cerebral artery occlusion or stenosis had a history of cerebral infarction. Individuals excluded from the study included patients who experienced a vascular event within 30 days of measurements, premenopausal women, and those with evidence of infection and/or malignant disease. The number of circulating CD34+ cells was quantified as described (Taguchi *et al*, 2007). In brief, blood samples (200 μl) were incubated with phycoerythrin-labeled anti-CD34 antibody, fluorescein isothiocyanate-labeled anti-CD45 antibody, 7-aminoactinomycin-D (7-AAD), and internal control (all of these reagents are in the Stem-Kit; BeckmanCoulter, Marseille, France). After incubation, samples were centrifuged, and supernatant was removed to obtain concentrated cell suspensions. 7-Aminoactinomycin-D-positive dead cells and CD45-negative cells were excluded, and the number of cells forming clusters characteristic of CD34+ cells (i.e., low side scatter and low-to-intermediate CD45 staining) was counted. The absolute number of CD34+ cells was calculated using the internal control. Mean cell number of duplicate measurements was used for quantitative analysis. Statistical comparisons among groups were determined using analysis of variance or χ^2 test. Individual comparisons were performed using a two-tailed unpaired Students' *t*-test or Mann–Whitney's *U*-test. Mean ± s.e. is shown.

Results

Enrolled individuals were divided into three groups: control subjects, patients with cerebral occlusion or severe stenosis, but without the presence of vessels with angiographic characteristics of moyamoya disease, and patients with angiographic evidence of moyamoya-like vessels. Baseline characteristics of the groups are shown in Table 1. The modified Rankin scale evaluation of patients with and without moyamoya-like vessels was 0.5 ± 0.5 and 1.3 ± 0.2, respectively ($P = 0.15$). Comparing these groups, there was a significant difference in the ratio of gender and treatment with aspirin between groups. However, no significant difference was observed in the number of circulating CD34+ cells in control group between genders (male, $n = 13$, CD34+ cells = 0.93 ± 0.10/μL; female, $n = 13$, CD34+ cells = 0.85 ± 0.11/μL: $P = 0.59$) and treatment with aspirin (aspirin (+), $n = 6$, CD34+ cells = 0.76 ± 0.12/μL; aspirin (−), $n = 20$, CD34+ cells = 0.93 ± 0.09/μL: $P = 0.26$), indicating mild and nonsignificant effects of gender and treatment with aspirin on the level of circulating CD34+ cells. In univariate analysis of control subjects, each cerebrovascular risk factor and treatment with statins showed no significant difference in the number of circulating CD34+ cells (data not shown).

A representative angiogram showing characteristics of moyamoya-like vessels is shown in Figures 1A and 1B. Angiographic moyamoya-like vessels were observed around the M1 portion of an occluded middle cerebral artery. Compared with a normal subject (Figure 1C) and patients without angiographic evidence of moyamoya-like vessels (Figure 1D), a remarkable increase in levels of

Table 1 Baseline characteristics

	Total	*Control*	*Major artery occlusion/stenosis*		P-*value for trend*
			Moyamoya (−)	*Moyamoya (+)*	
N	50	26	20	4	
Age, years	60.8 ± 1.1	60.5 ± 1.9	61.5 ± 1.0	59.3 ± 5.9	0.85
Male, *n* (%)	33 (66)	13 (50)	18 (90)	2 (50)	0.01
Risk factor, n (%)					
Hypertension	35 (70)	16 (62)	15 (75)	4 (100)	0.24
Hyperlipidemia	26 (52)	14 (54)	10 (50)	2 (50)	0.96
Diabetes mellitus	11 (22)	7 (27)	4 (20)	0 (0)	0.46
Smoking	15 (30)	7 (27)	8 (40)	0 (0)	0.25
Treatment, n (%)					
Ca channel blockers	20 (40)	10 (38)	8 (40)	2 (50)	0.91
β-Blockers	5 (10)	3 (11)	1 (5)	1 (25)	0.44
ACE inhibitor	7 (14)	4 (15)	2 (10)	1 (25)	0.70
ARB	12 (24)	5 (19)	5 (25)	2 (50)	0.40
Diuretics	4 (8)	2 (7)	1 (5)	1 (25)	0.40
Statin therapy	14 (28)	9 (34)	4 (20)	1 (25)	0.54
Aspirin	19 (38)	6 (23)	10 (50)	3 (75)	0.05
Ticlopidine	12 (24)	3 (11)	8 (40)	1 (25)	0.08

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin 2 receptor blocker.