Table 1: Characteristics of patients undergoing local intra-arterial thrombolysis

Age, mean (SD), yr	65 (13)
Men, %	80.8
Cardioembolic stroke, %	92.3
Atrial fibrillation, %	76.9
Occlusion of M1 segment, %	42.3
History of risk factors, %	
Hypertension	57.7
Diabetes mellitus	19.2
Hypercholesterolemia	19.2
Smoking	46.2
Alcohol	42.3
Time to admission, mean (SD), hr	0.96 (0.87)
Time to starting LIT, mean (SD), hr	3.78 (1.17)
Early CT sign, %	46.2
Previous anticoagulant therapy, %	26.9
Previous antiplatelet therapy, %	15.4
Symptomatic hemorrhage, %	0
Baseline NIHSS score, median (range)	18 (6–23)
NIHSS score after LIT, median (range)	
Immediately	15 (3–22)
24 hr later	12 (2–22)
1 month later	6 (0–16)
BI score at discharge, median (range)	95 (0–100)
mRS score at discharge, median (range)	2 (0–5)
Deaths, %	0
Hospital stay, mean (SD), days	50 (23)

Note.—LIT indicates local intra-arterial thrombolysis; NIHSS, National Institutes of Health Stroke Scale; BI, Barthel index; mRS, modified Rankin scale.

Results

Demographics and Clinical Data

The baseline characteristics of the 26 patients who underwent LIT are shown in Table 1. LIT was commenced 3.78 ± 1.17 hours after stroke onset. Hemorrhagic transformation in 11 patients (42.4%) was revealed by CT. However, no patients developed SICH or died. The median of the baseline NIHSS score was 18 (range, score of 6–23) at admission and became 6 (range, score of 0–16) 1 month later. Stroke recurred in two patients during their hospital stay. The median mRS score was 2 (range, score of 0–5), and the median Barthel index score was 95 (range, score of 0–100) at the time of discharge.

Outcome Data

Thirteen of 26 patients achieved good outcomes (mRS score, ≤2), and the other 13 had poor outcomes (mRS score, ≥3). The median baseline NIHSS scores were not different between the two groups (median score of 16 versus median score of 18). Patients with poor outcomes experienced no recurrence of stroke and no major complication, such as pneumonia or heart failure, during their hospital stay. The length of hospital stay was longer for patients with poor outcomes than for those with good outcomes (61 versus 38 days). Patients with good outcomes were younger (age 57.3 years ± 13.1 for good outcome group versus age 72.5 years ± 6.2 for poor outcome group), had hypertension less frequently (38.5% versus 76.9%), had an alcohol habit more frequently (61.5% versus 23.0%), M1 occlusion (61.5% versus 23.1%), good collaterals (61.5% versus 15.4%), and good recanalization (69.2% versus 30.7%). No differences were observed based on sex, baseline NIHSS scores, time to admission, time to LIT commencement, frequency of early signs revealed by CT, antiplatelet or anticoagulant therapy before stroke, hemorrhagic transformation on CT, blood pressure levels, or dose of urokinase or heparin between the two groups (Table 2). The antihypertensive agents were IV infused in four patients with good outcomes (31%) and in six patients with poor outcomes (46%). No differences were noted in arterial blood pressure after LIT between the two groups (Table 2). No patient received anticoagulants or antiplatelet agents for 24 hours after treatment.

Figure 1 shows the evolution of the NIHSS scores after LIT in patients with good and poor outcomes. The NIHSS scores of patients with good outcomes improved immediately after LIT. On the other hand, this immediate improvement was not observed for those with poor outcomes. The median NIHSS score was 9 (range, score of 3–19) for the good outcome group and 18 (range, score of 13–22) for the poor outcome group immediately after LIT ($P = .001$). The NIHSS scores of 11 patients (85%) with good outcomes improved immediately after LIT by ≥2. On the other hand, such an improvement was noted for only one patient with poor outcome. The differences in the median NIHSS scores between the two groups gradually increased with time. Thus, the NIHSS scores were 7 (range, score of 2–19) for the patients with good outcomes and 18 (range, score of 10–22) for the patients with poor outcomes after 24 hours ($P = .001$). One month later, the scores were 2 (range, score of 0–9) versus 10 (range, score of 6–18) ($P < .0001$). The Barthel index score in the good outcome group was 100 for all patients at discharge, whereas in the poor outcome group, the median Barthel index score was 40 (range, score of 0–90) ($P < .0001$). The median mRS score at discharge was 1 (range, score of 0–2) in the patients with good outcomes and 4 (range, score of 3–5) in the patients with poor outcomes ($P < .0001$).

Logistic Regression Analysis

Univariate analysis showed that the variables associated with good outcome (mRS score, ≤2) at discharge were age, good collaterals, occlusion of M1 segment, alcohol habit, no history of hypertension, high grade recanalization, and improvement of NIHSS score by ≥2 immediately after LIT (ΔNIHSS score, ≥2) (Tables 3 and 4). We selected factors in baseline characteristics, such as age, good collaterals, occlusion of M1 segment, alcohol habit, and nonhypertension, as independent factors for multivariate analysis with a logistic regression model for good outcome (Table 3, Model 1). The analyses showed no factors to be significant.

Table 2: Demographic and clinical variables for patients undergoing local intra-arterial thrombolysis

Variable	mRS Score ≤2 (n = 13)	mRS Score ≥3 (n = 13)	*P* Value
Age, mean (SD), yr	57 (13)	73 (6)	.0003
Men, %	84.6	76.9	>.9999
History of risk factors, %			
Hypertension	38.5	76.9	.047
Diabetes mellitus	15.4	23.0	>.9999
Hypercholesterolemia	23.1	15.4	>.9999
Smoking	61.5	30.8	.116
Alcohol	61.5	23.0	.047
Previous anticoagulant therapy, %	38.5	15.4	.378
Previous antiplatelet therapy, %	23.0	7.6	.593
Time to admission, mean (SD), hr	1.06 (0.95)	0.85 (0.81)	.519
Time to starting LIT, mean (SD), hr	3.81 (1.23)	3.75 (1.15)	.858
Time to end LIT, mean (SD), hr	4.86 (1.11)	4.85 (1.22)	.739
Baseline NIHSS score, median (range)	16 (6–22)	18 (9–23)	.757
Blood pressure at admission, mean (SD), mmHg			
Systolic	149 (32)	151 (23)	.741
Diastolic	86 (15)	83 (9)	.739
Early CT sign, %	53.8	38.5	.431
Occlusion of M1 segment, %	61.5	23.1	.047
Collateral flow (≥50%), %	61.5	15.4	.016
Dose of urokinase, mean (SD), $\times 10^4$ IU	40.6 (11.6)	36 (14.9)	.527
Dose of heparin, mean (SD), $\times 10^3$ IU	3.8 (2.8)	3.3 (2.1)	.723
Recanalization (≥50%), %	69.2	30.7	.049
Hemorrhagic transformation on CT, %	38.5	46.2	.691
Use of antihypertensive drug, %	31	46	.973
Blood pressure after LIT (with/without antihypertensive drug), mean (SD), mmHg			
Systolic	138 (10)	132 (23)	.275
Diastolic	73 (10)	76 (8)	.572
Hospital stay, mean (SD), days	38 (9)	61 (26)	.014

Note.—mRS indicates modified Rankin scale; LIT, local intra-arterial thrombolysis; NIHSS, National Institutes of Health Stroke Scale.

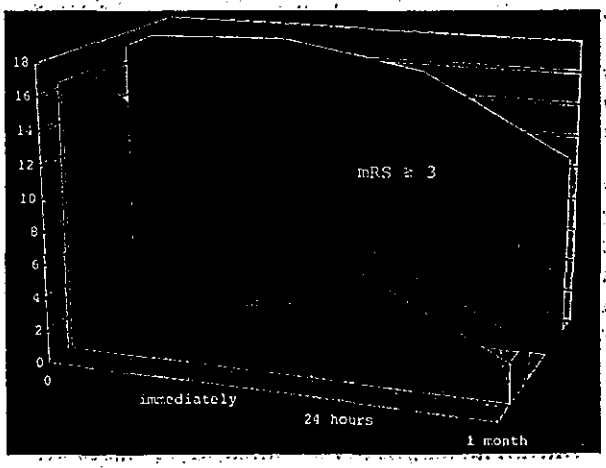

FIG 1. Progression of NIHSS scores of patients with good outcomes and those with poor outcomes.

In a Model 2 analysis, we added ΔNIHSS score ≥ 2 and high grade recanalization to factors of age and good collaterals that showed $P < .2$ in the Model 1 multivariate analysis as an independent factors (for multivariate analysis with a logistic regression model) for good outcome (Table 4). As a result, ΔNIHSS ≥ 2 was the only significant predictor for favorable outcome.

Discussion

The National Institute of Neurologic Disorders and Stroke rt-PA trial revealed that SICH occurred in the rt-PA group 10 times more often than in the placebo group (1). Other randomized controlled trials reported that SICH occurred in 8% to 20% of patients (2–6). In our study, in which LIT was performed only in patients with embolic MCA occlusion, no cases of SICH (95% confidence interval, 0.871–1) or death occurred. Levy et al (7) reported that a predictor of SICH was a high mean blood pressure before IV tissue plasminogen activator treatment. Kidwell et al (8) found that in patients who had undergone LIT, the NIHSS score in the SICH group was higher than in the non-SICH group (mean NIHSS scores, 20 in SICH versus 15 in non-SICH). Other predictors of hemorrhagic transformation were longer time to recanalization, lower platelet count, and higher glucose level. The inclusion and exclusion criteria for LIT in our study were similar to those of the National Institute of Neurologic Disorders and Stroke rt-PA trial except for the time window, and we strictly followed the American Heart Association guidelines (3). As a result, patients with excessively high baseline NIHSS scores (scores > 29) were excluded from the study. In our study, 50% of the patients had mRS scores ≤2 at discharge, whereas 40% of the patients in the Prolyse in Acute Cerebral Thromboembolism II study had

Table 3: Logistic regression analysis for pretreatment predictors of favorable outcome in patients undergoing local intra-arterial thrombolysis (Model 1)

	Univariate			Multivariate		
Variable	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Age <60 yr	5.1	1.02–30.06	.027	7.8	0.49–308.36	.182
Good collaterals	8.8	1.55–74.68	.016	6.1	0.53–102.44	.166
Occlusion of M1 segment	5.3	1.04–33.91	.047	5.4	0.41–139.85	.218
Alcohol habit (+)	5.3	1.04–33.91	.047	3.5	0.22–95.49	.377
Hypertension (−)	5.3	1.04–33.91	.047	2.3	0.19–29.67	.483

Note.—OR indicates odds ratio; CI, confidence interval.

Table 4: Logistic regression analysis for predictors of favorable outcome for patients undergoing local intra-arterial thrombolysis (Model 2)

	Univariate			Multivariate		
Variable	OR	95% CI	*P* Value	OR	95% CI	*P* Value
ΔNIHSS score ≥2	65.9	7.46–1656.23	.001	38.8	2.40–2112.46	.024
High grade recanalization	10.3	1.37–216.38	.049	18.0	0.59–1538.90	.126
Age <60 yr	5.1	1.02–30.06	.027	7.7	0.32–296.83	.265
Good collaterals	8.8	1.55–74.68	.016	6.0	0.18–522.90	.261

Note.—OR indicates odds ratio; CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale.
*ΔNIHSS score was calculated by subtracting immediate NIHSS score from baseline NIHSS score.

mRS scores ≤2 at day 90. Therefore, strict application of the criteria for the management of patients with LIT seems to be essential to obtain favorable outcomes and to avoid SICH. Additionally, antihypertensive medicine was aggressively infused through an IV route in patients who had high systolic blood pressure >180 mmHg after LIT. None of our patients had blood pressure >180 mmHg after antihypertensive treatment. It was suggested that control of blood pressure after LIT was also an important factor against SICH.

Bendszus et al (12) reported artery factors related to good outcomes in patients who had undergone LIT of MCA and internal carotid artery occlusion. They noted that presence of leptomeningeal collaterals and successful recanalization after LIT correlated with good outcome, whereas the interval from stroke onset to LIT was not related to it. However, their series had a 10% incidence of SICH. Arnold et al (13) reported that a low NIHSS score at admission and good vessel recanalization were independently associated with good outcome, and SICH occurred in 7% of patients. In the Prolyse in Acute Cerebral Thromboembolism II study, it was reported that outcome is associated with history of cerebrovascular disease, hypertension, and diabetes (14).

In our study, as in previous reports, the good outcome group was younger and had a higher rate of good collaterals and higher grade recanalization than the poor outcome group. In addition, the good outcome group had few cases of hypertension, as in the Prolyse in Acute Cerebral Thromboembolism II study, and alcohol habit and M1 occlusion were frequent. Aronowski et al (15) reported that a low dose of ethanol plus caffeine reduced infarct volume in a rat model of transient MCA occlusion. Although the optimal dose of alcohol was unknown, our result suggested that alcohol was a neuroprotective factor in the thrombolytic therapy. It seems very interesting that in our study, 62% of the good outcome group had M1 occlusion, compared with only 23% of the group with mRS ≥3. Gönner et al (16) indicated that good outcomes were achieved in 57% of patients with occlusion of M1 and perforators. On the other hand, all patients who had M1 occlusion without involving perforators or an M2 occlusion achieved good outcomes (100%). All our patients had M1 distal occlusions without perforator involvement. Although the rate of recanalization was not significantly different between patients with M1 occlusion and those with M2 occlusion, the patients with M1 occlusion had good collaterals (90%) compared with the patients with M2 occlusion (6%). The grade of collaterals may be more important for later outcome than the site of arterial occlusion.

No study has reported in detail on the evolution of NIHSS scores after LIT. In the good outcome group, the NIHSS score improved immediately after LIT and then continued to improve gradually. On the other hand, in the poor outcome group, the NIHSS score remained stable for nearly 24 hours (Fig 1). Multiple logistic regression analysis revealed that ΔNIHSS immediately after LIT was the only independent factor for good outcome. Thus, later outcome can be predicted by a change in NIHSS score (ΔNIHSS) immediately after LIT.

The present study had some limitations. First, it was an observational study. We cannot compare our results with those of patients who did not undergo LIT. Therefore, the effect of LIT for MCA occlusion is unclear. Second, our study size was small, so care must be taken in the interpretation of the analyses.

Conclusion

Strict criteria should be applied in selecting patients with MCA occlusion for LIT to avoid SICH. Favorable outcome at a later time or discharge may be predicted by improvement in NIHSS score immediately after LIT. These observations should be confirmed in large prospective randomized controlled trials.

References

1. 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
2. Hacke W, Kaste M, Fieschi C, et al. **Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke: The European Cooperative Acute Stroke Study (ECASS).** *JAMA* 1995;274:1017–1025
3. Albers GW, Clark WM, Madden KP, et al. **ATLANTIS trial: results for patients treated within 3 hours of stroke onset: alteplase thrombolysis for acute noninterventional therapy in ischemic Stroke.** *Stroke* 2002;33:493–495
4. Adams HP Jr, Brott TG, Furlan AJ, et al. **Guidelines for thrombolytic therapy for acute Stroke: a supplement to the guidelines for the management of patients with acute ischemic stroke: a statement for healthcare professionals from a Special Writing Group of the Stroke Council, American Heart Association.** *Stroke* 1996;27:1711–1718
5. Furlan A, Higashida R, Wechsler L, et al. **Intra-arterial prourokinase for acute ischemic stroke: The PROACT II study: a randomized controlled trial: prolyse in acute cerebral thromboembolism.** *JAMA* 1999;282:2003–2011
6. Hacke W, Kaste M, Fieschi C, et al. **Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II): Second European-Australasian Acute Stroke Study Investigators.** *Lancet* 1998;352:1245–1251
7. Levy DE, Brott TG, Haley EC Jr, et al. **Factors related to intracranial hematoma formation in patients receiving tissue-type plasminogen activator for acute ischemic stroke.** *Stroke* 1994;25:291–297
8. Kidwell CS, Saver JL, Carneado J, et al. **Predictors of hemorrhagic transformation in patients receiving intra-arterial thrombolysis.** *Stroke* 2002;33:717–724
9. Moulin T, Cattin F, Crepin-Leblond T, et al. **Early CT signs in acute middle cerebral artery infarction: predictive value for subsequent infarct locations and outcome.** *Neurology* 1996;47:366–375
10. Mori E, Yoneda Y, Tabuchi M, et al. **Intravenous recombinant tissue plasminogen activator in acute carotid artery territory stroke.** *Neurology* 1992;42:976–982
11. Yamaguchi T, Hayakawa T, Kikuchi H, et al. **Intravenous tissue plasminogen activator ameliorates the outcome of hyperacute embolic stroke.** *Cerebrovasc Dis* 1993;3:269–272
12. Bendszus M, Urbach H, Ries F, et al. **Outcome after local intra-arterial fibrinolysis compared with the natural course of patients with a dense middle cerebral artery on early CT.** *Neuroradiology* 1998;40:54–58
13. Arnold M, Schroth G, Nedeltchev K, et al. **Intra-arterial thrombolysis in 100 patients with acute stroke due to middle cerebral artery occlusion.** *Stroke.* 2002;33:1828–1833
14. Wechsler LR, Roberts R, Furlan AJ, et al. **Factors influencing outcome and treatment effect in PROACT II.** *Stroke* 2003;34:1224–1229
15. Aronowski J, Strong R, Shirzadi A, et al. **Ethanol plus caffeine (caffeinol) for treatment of ischemic stroke: preclinical experience.** *Stroke* 2003;34:1246–1251
16. Gönner F, Remonda L, Mattle H, et al. **Local intra-arterial thrombolysis in acute ischemic stroke.** *Stroke* 1998;29:1894–1900

AJNR Am J Neuroradiol 25:1680–1684, November/December 2004

Transcranial Color-Coded Real-Time Sonographic Criteria for Occlusion of the Middle Cerebral Artery in Acute Ischemic Stroke

Toshiyasu Ogata, Kazumi Kimura, Makoto Nakajima, Kouichi Ikeno, Hiroaki Naritomi, and Kazuo Minematsu

BACKGROUND AND PURPOSE: **Transcranial color-coded real-time sonography (TCCS) is a useful tool to evaluate disease of the middle cerebral artery (MCA). This study was undertaken to identify TCCS criteria for the diagnosis of MCA stem and MCA branch occlusions.**

METHODS: **TCCS and digital subtraction angiography were performed in 55 consecutive patients with acute stroke: 10 with MCA stem occlusion, the MO group; eight with MCA branch occlusion, the MB group; and 37 with nonocclusive lesions, the control group. We measured the end-diastolic velocity (EDV) of the bilateral MCA stems and calculated the end-diastolic ratio by dividing the EDV of the unaffected side by that of the affected side.**

RESULTS: **EDV was highest in the control group, and end-diastolic ratio was highest in the MO group. An EDV of >25 cm/s indicated a nonocclusive lesion in the MCA, with a positive predictive value of 98.4%, a negative predictive value of 81.0%, and an accuracy of 93.9%. An EDV of ≤25 cm/s with an end-diastolic ratio of <2.7 indicated an MCA branch occlusion with a positive predictive value of 85.7%, a negative predictive value of 97.2%, and an accuracy of 95.3%. An EDV of ≤25 cm/s with an end-diastolic ratio of ≥2.7 indicated MCA stem occlusion with a positive predictive value of 100%, a negative predictive value of 100%, and an accuracy of 100%.**

CONCLUSION: **We developed TCCS criteria for the diagnosis of MCA diseases. MCA flow velocity detected by means of TCCS can help identify MCA stem occlusion as well as MCA branch occlusion.**

In the early 1990s, transcranial color-coded real-time sonography (TCCS) was introduced as a new method for imaging the basal cerebral arteries (1–3). TCCS is a noninvasive, easy-to-repeat, diagnostic technique that is widely used for the evaluation of cerebral hemodynamics. As a result of combining the B-mode facility and the color-coded Doppler facility, the brain vessels can be clearly displayed. Furthermore, since the angle of insonation can be measured and corrected for, one can obtain velocity measurements that are closer to true values (4, 5). Thus, TCCS is a useful tool in evaluating vascular diseases of the middle cerebral arteries (MCAs), particularly in patients with ischemic stroke.

However, only a few reports have described the diagnosis of MCA stem occlusion with TCCS (6, 7). In fact, TCCS criteria for MCA occlusion, particularly MCA branch occlusion, have not yet been established. MCA branch occlusion frequently occurs in patients with acute ischemic stroke. Therefore, it is important to be able to evaluate the MCA branch occlusion as well as the MCA stem occlusion when one is deciding on a patient's therapy.

Peripheral resistance, which is found after the point of measurement, is thought to reflect blood flow velocity; the higher peripheral resistance, the lower the blood flow velocity. Therefore, we hypothesized that the blood velocity in the MCA stem of patients with MCA branch occlusion is lower than that in patients without MCA occlusion and that it is higher than that of patients with MCA stem occlusion. The aim of the current study was to establish TCCS criteria for determining the specific sites of MCA occlusion.

Received November 11, 2003; accepted after revision March 25, 2004.

From the Department of Medicine, Cerebrovascular Division, National Cardiovascular Center, Osaka, Japan.

Supported in part by Research Grants for Cardiovascular Diseases (14A-1) and Comprehensive Research on Aging and Health from the Ministry of Health and Welfare of Japan.

Address reprint requests to Toshiyasu Ogata, MD, Department of Medicine and Clinical Science, Graduate School of Medical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812-8582, Japan.

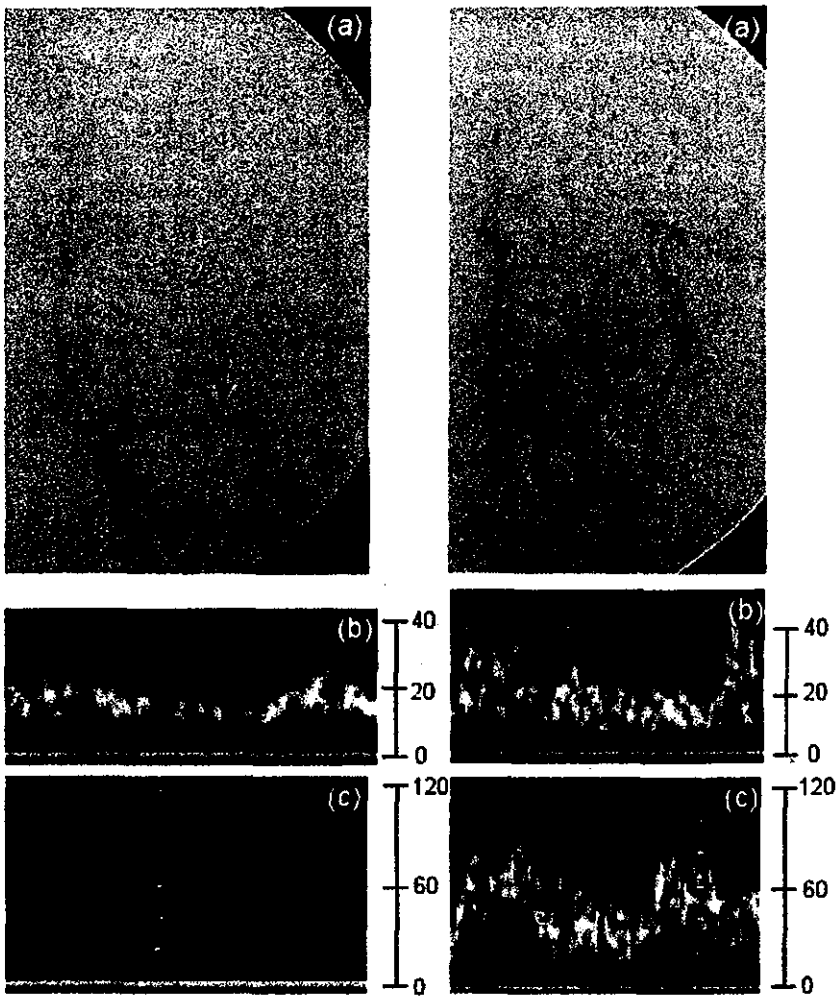

FIG 1. Left anteroposterior carotid angiograms *in representative cases* evaluated with cerebral angiography and TCCS. Y axis is represented blood flow velocity (cm/s). *Left* image shows occlusion of the horizontal portion of the left MCA (*a*). Occlusion site overlies the external carotid artery branch. Doppler waveforms of the left (*b*) and right (*c*) MCAs show EDVs of 14.5 and 49.9 cm/s, respectively. *Right* image shows occlusion of the branch in the left MCA (*a*). Doppler waveforms of the left (*b*) and right (*c*) MCAs show EDVs of 18.2 and 44.6 cm/s, respectively.

Methods

We prospectively performed TCCS in 66 consecutive patients with acute stroke who underwent intra-arterial digital subtraction angiography (DSA). TCCS examinations were performed 24 hours before and after the intra-arterial DSA study. Eleven patients (three with occlusion or a severe stenosis in internal carotid artery, six with MCA stem stenosis, and two with proximal MCA occlusion) were excluded from this study. Patients with proximal MCA occlusion were excluded because TCCS could not display the flow signal of the MCA, and thus the flow velocity could not be measured. Therefore, 55 patients were enrolled. Their stroke subtypes were as follows: two patients had a lacunar stroke, 10 had atherothrombotic stroke, 20 had cardioembolic stroke, one had a transient ischemic attack, 18 had other types of ischemic stroke, three had hemorrhagic stroke, and one had amourosis fugax.

The study protocol followed all principles outlined in the Declaration of Helsinki. Selective angiography was performed by using biplane DSA (Angio Rex Super-G and DFP-2000A; Toshiba, Tokyo, Japan). All examinations were performed by means of transbrachial or transfemoral catheterization in accordance with the Seldinger method. Standard anteroposterior and lateral images were routinely obtained.

According to the DSA results, we assigned the patients as follows: Patients with an MCA stem occlusion were the MO group, patients with an MCA branch occlusion were the MB group, and patients with no occlusive or stenotic MCA lesions were the control group.

TCCS was performed by using a unit (Sonos 5500; Philips Medical Systems, Japan, Tokyo) with a 1.0–3.0-MHz sector scan. The transtemporal acoustic window was used to visualize the MCA stem in real time by using color signals. We obtained color Doppler flow images and measured flow velocity at the MCAs. Patients were examined first in the left lateral decubitus position and then in the right lateral decubitus position. Particular care was taken to identify an appropriate straight-vessel segment of the MCA by means of tilting, rotating, or shifting the transducer. A 1.9-mm, range-gated, pulsed Doppler sample volume was used to measure the blood flow velocity in the MCA stem. The sample volume was moved slowly from the proximal to the distal position along the horizontal segment of the MCA and displayed as a color flow image on B-mode images. We chose the measured point where the blood flow velocity was the highest. Then we measured the mean end-diastolic velocity (EDV) over five consecutive cardiac cycles. Angle correction was applied when the correction angle did not exceed 60°. Furthermore, the side-to-side ratio of the end-diastolic flow velocity (end-diastolic ratio) was calculated by dividing the velocity of the unaffected side by that of the affected side in the MO and MB groups. We also detected the end-diastolic ratio of patients in the control group by dividing the higher MCA velocity by the lower one.

The age and blood flow velocity data for each group were expressed as the mean ± SD. Statistical comparisons of velocity differences within each group were performed by using one-way factorial analysis of variance and then Scheffé multiple comparison tests. Sensitivity-specificity curve analysis was applied to obtain cutoff values for EDV to distinguish the MO or MB group from the control group and for the end-diastolic ratio to differ-

entiate the MO group from the MB group. A P value of $< .05$ was accepted as indicating a significant difference.

Results

We performed intra-arterial DSA in 55 patients (46 men and nine women; mean age, 63.8 ± 13.1 years). TCCS depicted bilateral MCA flow signals in 43 patients. However, TCCS depicted only unilateral MCA flow signal intensity in the other 12 patients. Consequently, the EDV of 98 vessels was measured. Occlusive lesions were present in 10 patients with a unilateral mid-to-distal occlusion of MCA stem, seven patients with a unilateral MCA branch occlusion, one patient with bilateral MCA branch occlusions, and 37 patients without a significant occlusion or stenosis. Thus, the groups consisted of patients with an occlusion of the MCA stem (MO group, $n = 10$), those with an occlusion of the MCA branch (MB group, $n = 8$), and patients with no occlusive lesions (control group, $n = 37$).

We did not detect MCA flow on the right side in the patient with bilateral MCA branch occlusions. Typical waveforms obtained in the MO and MB groups are shown in Figure 1.

The end-diastolic ratio was calculated for all patients in whom bilateral MCAs were detected. Scattergrams of the EDV and the end-diastolic ratio for each group are shown in Figure 2. EDV was significantly higher in the control group (40.5 ± 11.5 cm/s) than in the MO group (12.2 ± 3.6 cm/s, $P < .001$) or MB group (19.6 ± 4.8 cm/s, $P < .001$). The end-diastolic ratio (4.2 ± 1.5) of the MO group was significantly greater than that of the MB group (1.8 ± 0.5, $P < .001$) or control group (1.2 ± 0.1, $P < .001$).

On sensitivity-specificity curve analysis, the optimal threshold value of EDV for differentiating the MO and MB groups from the control group was 25 cm/s (Fig 3A). A positive predictive value of 81.0%, a negative predictive value of 98.4%, and an accuracy of 93.9% were calculated for the optimal threshold value. The optimal threshold value of the end-diastolic ratio for discriminating the MO group from the MB group was 2.7 (Fig 3B), with a positive predictive value of 100%, a negative predictive value of 100%, and an accuracy of 100%.

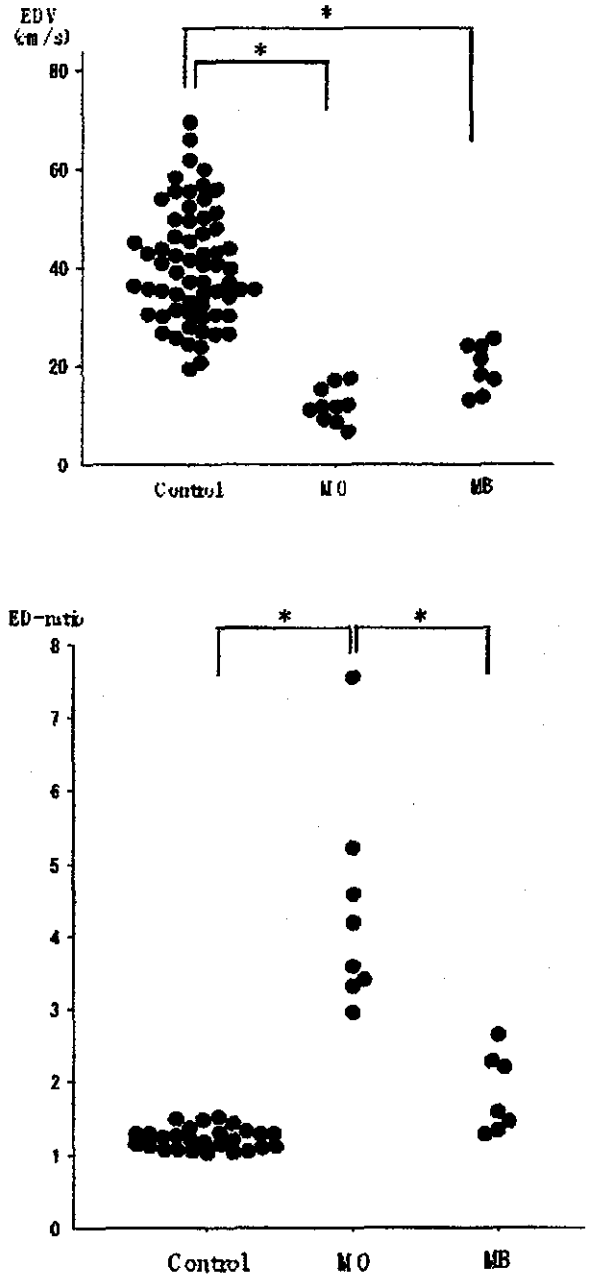

FIG 2. Scattergrams. *Top*, Mean EDVs (±2 SDs) for the control, MO, and MB groups are 40.5 ± 11.5, 12.2 ± 3.6, and 19.6 ± 4.8, respectively ($P < .001$, Scheffé test). *Bottom*, Mean end-diastolic ratios (±2 SDs) for the control, MO, and MB groups are 1.2 ± 0.1, 4.2 ± 1.5, and 1.8 ± 0.5, respectively ($P < .001$, Scheffé test).

Discussion

To our knowledge, this is the first study to develop TCCS criteria for diagnosing MCA stem occlusion and MCA branch occlusion. Kimura et al (7) reported that the end-diastolic ratio of patients with an MCA stem occlusion might increase to >1.9. In the present study, the end-diastolic ratio in the MO group was higher than 1.9, and this is compatible with the previous report by Kimura et al. Because they did not report TCCS criteria for determining MCA branch occlusion, the results of our MB group cannot be compared with their results.

Sensitivity-specificity curve analysis demonstrated an optimal threshold EDV value of 25 cm/s for differentiating MO and MB patients from control patients. In the MO and MB groups, 17 (94.4%) of 18 patients had an EDV <25 cm/s. However, of 37 patients in the control group, four (10.8%) had an EDV < 25 cm/s. Therefore, if the EDV is <25 cm/s, one cannot always identify the group (MO, MB, or control) to which the patient belongs. This is a limitation of our study. We have already reported that the end-diastolic ratio of control group patients was <1.9, even if EDV was under 25 cm/s (7). Therefore, the

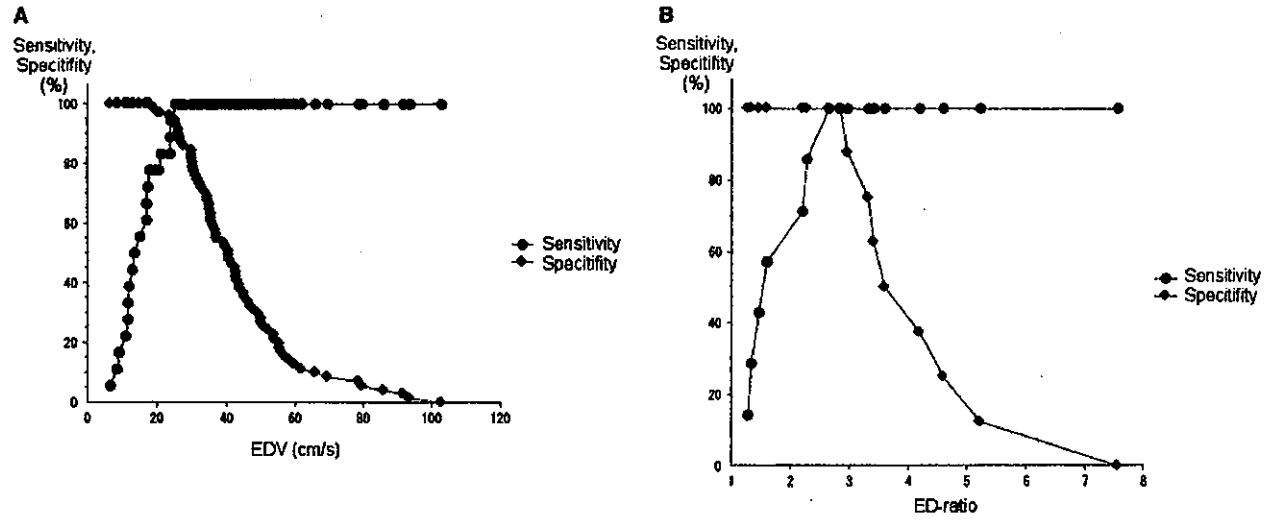

FIG 3. Sensitivity-specificity curves.
A, Predicting MO or MB by EDV. Optimal threshold value for EDV is 25 cm/s.
B, Differentiating MO from MB by the end-diastolic ratio. Optimal threshold value for the ratio is 2.7.

end-diastolic ratio should be useful in deciding if patients with EDV of ≤25 cm/s have an occlusive MCA lesion.

In the present study, we observed no differences in the end-diastolic ratio between the MB group and the control group. Therefore, the end-diastolic ratio alone is insufficient to diagnose an MCA branch occlusion. However, an end-diastolic ratio of 2.7 perfectly distinguished the MO group from the MB group. By using both the EDV and the end-diastolic ratio, we could distinguish the MB group from the control and MO groups. These results can be explained by the differences in the peripheral resistances among the MO, MB, and control groups. We conclude that the TCCS criteria of an EDV of ≤25 cm/s and an end-diastolic ratio of <2.7 indicates an MCA branch occlusion and that an EDV of ≤ 25 cm/s and an end-diastolic ratio of ≥2.7 indicates an MCA stem occlusion. The diagnostic algorithm for MCA stem and branch occlusion is shown in Figure 4.

A few patients could not be examined because of inadequate insonation windows during TCCS. The failure rate increased with age and was higher in women because of the higher prevalence of temporal hyperosteosis (8). Furthermore, the detection rate of intracranial artery flow signal intensity by using transcranial Doppler imaging is lower in Japanese patients than in white patients (9). Contrast agents can increase the detection rate of the MCA with TCCS (8, 10–13). Therefore, use of a contrast agent may help in diagnosing MCA diseases if the MCA flow cannot be detected with conventional TCCS.

When intravenous thrombolysis with tissue plasminogen activator (t-PA) is given to ischemic stroke patients within 3 hours of stroke onset, long-term outcomes improve (14). The Prolyse in Acute Cerebral Thromboembolism (PROACT) II study (15) demonstrated a significant benefit from treatment with intra-arterial prourokinase in patients with MCA occlusion treated within 6 hours of stroke onset.

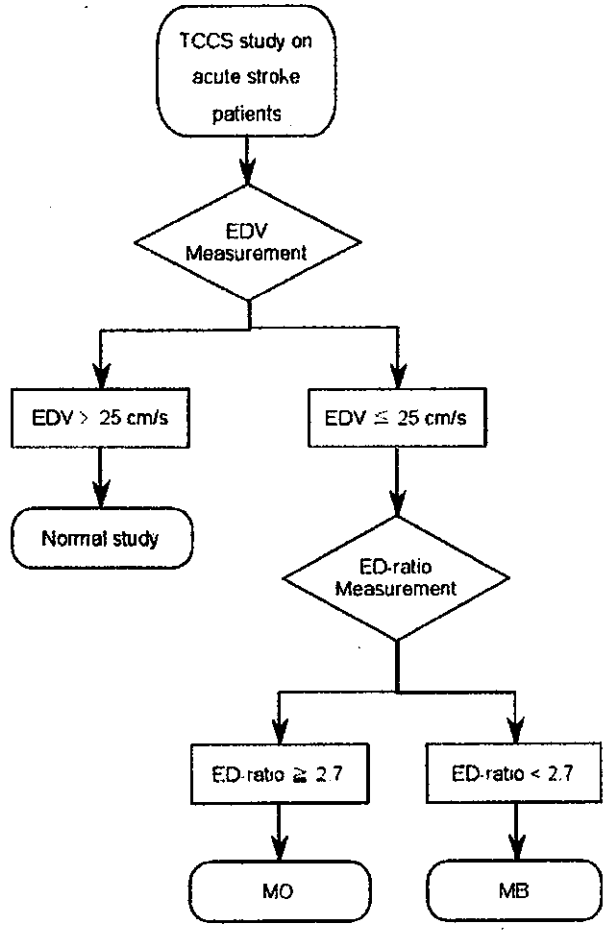

FIG 4. Algorithm for diagnosing MO and MB by using the EDV and end-diastolic ratio on TCCS.

Therefore, our TCCS criteria for MCA diseases may be useful in determining whether we perform the intra-arterial or venous thrombolysis in patents with hyperacute stroke. Furthermore, Eggers et al (16)

reported that the use of sonography with t-PA therapy improved outcomes in patients with hyperacute ischemic stroke. Therefore, in the near future, TCCS may be useful not only as a diagnostic tool but also as a treatment in patients with MCA disease.

Conclusion

To our knowledge, we are the first group to develop TCCS criteria for diagnosing MCA stem occlusion and MCA branch occlusion. TCCS is a useful tool in the assessment of MCA diseases in patients with acute stroke.

References

1. Bogdahn U, Becker G, Winkler J, Greiner K, Perez J, Meurers B. **Transcranial color-coded real-time sonography in adults.** *Stroke* 1990;21:1680–1688
2. Martin PJ, Evans DH, Naylor AR. **Transcranial color-coded sonography of the basal cerebral circulation: reference data from 115 volunteers.** *Stroke* 1994;25:390–396
3. Tsuchiya T, Yasaka M, Yamaguchi T, Kimura K, Omae T. **Imaging of the basal cerebral arteries and measurement of blood velocity in adults by using transcranial real-time color flow Doppler sonography.** *AJNR Am J Neuroradiol* 1991;12:497–502
4. Martin PJ, Evans DH, Naylor AR. **Measurement of blood flow velocity in the basal cerebral circulation: advantages of transcranial color-coded sonography over conventional transcranial Doppler.** *J Clin Ultrasound* 1995;23:21–26
5. Klotzsch C, Popescu O, Sliwka U, Mull M, Noth J. **Detection of stenoses in the anterior circulation using frequency-based transcranial color-coded sonography.** *Ultrasound Med Biol* 2000;26:579–584
6. Kenton AR, Martin PJ, Abbott RJ, Moody AR. **Comparison of transcranial color-coded sonography and magnetic resonance angiography in acute stroke.** *Stroke* 1997;28:1601–1606
7. Kimura K, Hashimoto Y, Hirano T, Uchino M, Ando M. **Diagnosis of middle cerebral artery occlusion with transcranial color-coded real-time sonography.** *AJNR Am J Neuroradiol* 1996;17:895–899
8. Postert T, Braun B, Meves S, et al. **Contrast-enhanced transcranial color-coded sonography in acute hemispheric brain infarction.** *Stroke* 1999;30:1819–1826
9. Itoh T, Matsumoto M, Handa N, et al. **Rate of successful recording of blood flow signals in the middle cerebral artery using transcranial Doppler sonography.** *Stroke* 1993;24:1192–1195
10. Gerriets T, Seidel G, Fiss I, Modrau B, Kaps M. **Contrast-enhanced transcranial color-coded duplex sonography: efficiency and validity.** *Neurology* 1999;52:1133–1137
11. Goertler M, Kross R, Baeumer M, et al. **Diagnostic impact and prognostic relevance of early contrast-enhanced transcranial color-coded duplex sonography in acute stroke.** *Stroke* 1998;29:955–962
12. Baumgartner RW, Arnold M, Gonner F, et al. **Contrast-enhanced transcranial color-coded duplex sonography in ischemic cerebrovascular disease.** *Stroke* 1997;28:2473–2478
13. Zunker P, Wilms H, Brossmann J, Georgiadis D, Weber S, Deuschl G. **Echo contrast-enhanced transcranial ultrasound: frequency of use, diagnostic benefit, and validity of results compared with MRA.** *Stroke* 2002;33:2600–2603
14. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. **Tissue plasminogen activator for acute ischemic stroke: the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group.** *N Engl J Med* 1995;333:1581–1587
15. Furlan A, Higashida R, Wechsler L, et al. **Intra-arterial prourokinase for acute ischemic stroke: the PROACT II study—a randomized controlled trial. Prolyse in Acute Cerebral Thromboembolism.** *JAMA* 1999;282:2003–2011
16. Eggers J, Koch B, Meyer K, Konig I, Seidel G. **Effect of ultrasound on thrombolysis of middle cerebral artery occlusion.** *Ann Neurol* 2003;53:797–800

Original Paper

Cerebrovascular Diseases

Cerebrovasc Dis 2004;18:166–173
DOI: 10.1159/000079737

Received: July 28, 2003
Accepted: February 26, 2004
Published online: July 13, 2004

Link between Linear Hyperintensity Objects in Cerebral White Matter and Hypertensive Intracerebral Hemorrhage

Masahiko Hiroki[a] · Kotaro Miyashita[b] Hiroshi Oe[b] Shigetoshi Takaya[a]
Shunsaku Hirai[c] Hidenao Fukuyama[a]

[a]Human Brain Research Center, Kyoto University Graduate School of Medicine, Kyoto, [b]Cerebrovascular Division, Department of Medicine, National Cardiovascular Center, Suita, and [c]Department of Neurology, Tokyo Metropolitan Neurological Hospital, Fuchu, Japan

Key Words
Linear hyperintensity objects · Cerebral white matter · MRI · Intracerebral hemorrhage · Hypertension

Abstract
Background: We retrospectively studied the relationship between linear hyperintensity objects (LHOs) on T_2-weighted magnetic resonance images (MRI) in the cerebral white matter and the occurrence of hypertensive intracerebral hemorrhage (HIH). ***Methods:*** Forty-nine hypertensive patients with a fixed imaging condition MRI were classified into three groups: HIH (n = 17), ischemic stroke due to hypertensive vasculopathy (n = 19), and hypertension only (n = 13). After assessing clinical and radiological background information among these groups and the reliability of LHO measurements, polynomial logistic regression analysis was used to identify the factors relating to HIH. ***Results:*** HIH had a significantly higher LHO number (p = 0.002) and larger diameter (p = 0.007). The LHO number showed an excellent interrater (κ = 0.91, 95% CI = 0.87–0.94, SEM = 6.2%) and intrarater reliability (κ = 0.95, 95% CI= 0.92–0.97, SEM = 4.8%), and was the most significant independent indicator of HIH (OR = 1.29, 95% CI = 1.05–1.60, p = 0.017). The number of microbleeds was an additional indicator (OR = 3.73, 95% CI = 1.10–12.65, p = 0.034). ***Conclusions:*** LHOs are closely linked to HIH. A prospective, longitudinal study is needed to clarify whether LHOs can predict HIH.

Introduction

Hypertensive intracerebral hemorrhage (HIH) is widely regarded as the deadliest of stroke subtypes. Unfortunately, there is no comprehensive method for detection of HIH in most hypertensive patients, except for microbleeds on T_2- or T_2*-weighted MRIs of patients with ischemic stroke or who take antithrombotic medication [1–4]. We recently found that linear hyperintensity objects (LHOs) on T_2-weighted MRIs of cerebral white matter are more prominent depending on the severity of hypertension, and may be based on both hypertensive arteriolar vasculopathy of the white matter medullary artery and its dilated perivascular spaces [5, 6]. Notably, most of the patients with prominent LHOs were noted to have suffered from HIH (fig. 1). Therefore, it is speculated that

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/04/0182–0166$21.00/0

Accessible online at:
www.karger.com/ced

Masahiko Hiroki, MD, PhD
Massachusetts General Hospital NMR Center
Building 149, 13th Street, Mailcode 149-2301
Charlestown, MA 02129-2060 (USA)
Tel. +1 617 726 3914, Fax +1 617 726 7422, E-Mail CY101752@nifty.com

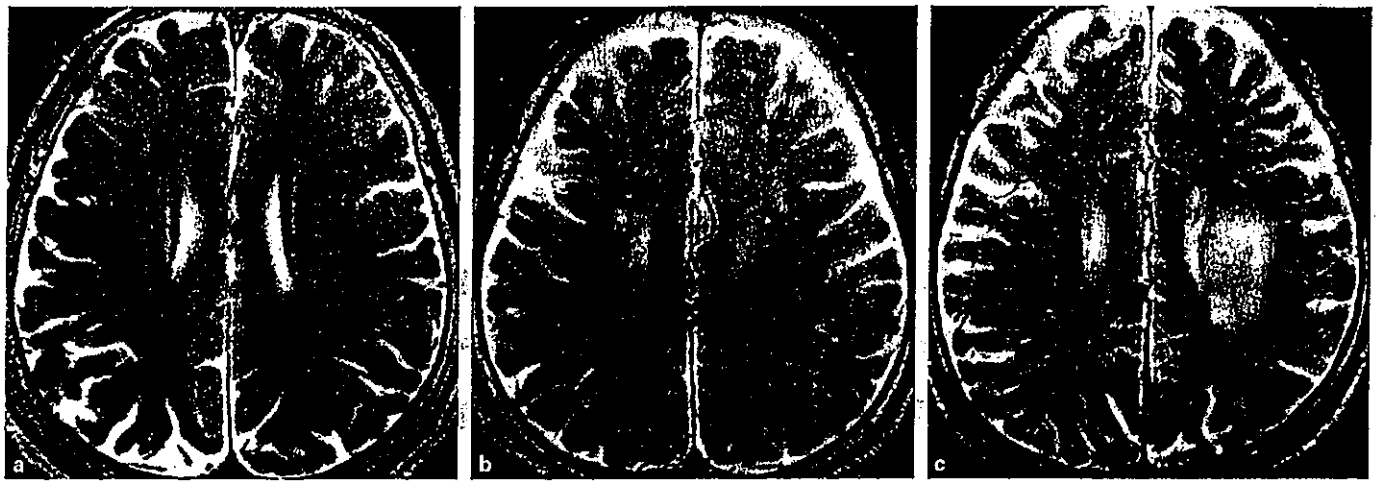

Fig. 1. Typical LHOs in hypertension-only (**a**), ischemic stroke due to hypertensive vasculopathy (**b**), and HIH (**c**). Case A: A 48-year-old patient had hypertension of 12 years' duration and no organic lesions in any area of the brain. Subtle LHOs are seen in the cerebral white matter. Case B: A 53-year-old patient had hypertension of 13 years' duration and multiple small infarcts in the basal ganglia and corona radiata. LHOs are less apparent. Case C: A 37-year-old patient had hypertension of 8 years' duration and left putaminal hemorrhage. Prominent LHOs are seen in the bilateral cerebral white matter.

LHOs and HIH may share a common pathological background as hypertensive microangiopathy, and that LHOs may be a promising indicator of HIH. Since the detection of details such as LHOs strongly depends on the imaging condition [7], we retrospectively and cross-sectionally studied hypertensive patients who underwent magnetic resonance imaging (MRI) with a fixed-imaging condition, focusing on the reliability of our manual evaluation of LHOs, and on the relationship between the severity of LHOs and the presence of HIH, taking the clinical and radiological backgrounds into consideration.

Materials and Methods

Subject Selection and Classification

A brain MRI with a fixed-imaging condition was performed on 274 consecutive patients admitted to the Department of Neurology at the Tokyo Metropolitan Neurological Hospital between May 1996 and April 1998. Patients with various brain disorders routinely underwent a brain MRI in our Department. The clinical diagnosis of the 274 patients was as follows: cerebrovascular disease in 105, infectious disease in 19, spinocerebellar degeneration in 18, motor neuron disease in 18, multisystem atrophy in 16, Parkinson's disease in 12, multiple sclerosis in 12, progressive supranuclear palsy in 7, and other disorders in the remaining 67 patients. From this group of patients, we selected all of the 87 patients with hypertension and grouped them into the following: HIH, ischemic stroke, transient ischemic attack, and hypertension only (fig. 2). Excluding patients whose MRIs were missing or had severe motion artifacts, 17 patients were selected for the HIH, 38 patients for the ischemic stroke, and 19 patients for the hypertension-only groups. Based on the WHO classification of severity of hypertensive organopathy in brain, heart, optic fundi, and kidney [8], we selected 31 patients with ischemic stroke due to hypertensive vasculopathy from the ischemic stroke group. Finally, to match the HIH patients for age, gender, duration of hypertension, and left ventricular hypertrophy, we grouped 19 patients into ischemic stroke due to hypertensive vasculopathy and 13 patients into hypertension only.

The diagnosis of HIH was made using all three clinical presentations including hypertensive systemic organopathy, MRI, and catheter or MR angiogram, in order to exclude non-HIH. Hemorrhage due to cerebral amyloid angiopathy was not included in the HIH group. Hematoma was located in the thalamus in 9 patients, putamen in 3, pons in 2, and the lobar region in 3. Ventricular extension was found in 1 case and recurrent hemorrhage in another. Mean hematoma volume, calculated by the ABC/2 method [9] with a CT scan of ≤ 6 h from onset, was 17.2 cm^3 (range: 1.4–69.0, n = 15; a CT scan could not be obtained from 2 patients). None of these patients were treated with sympatomimetic agents or took illegal drugs. Aspirin was prescribed for an HIH patient with prior cerebral ischemic stroke. The group with ischemic stroke due to hypertensive vasculopathy included either atherothrombotic or lacunar infarction classified according to the criteria of National Institute of Neurological Disorders and Stroke [10]. The hypertension-only group was defined as having both a history of hypertension and no asymptomatic hemorrhages or infarcts on MRI. Neurological diagnoses of the hypertension-only group were as follows: spinocerebellar degeneration in 2, Creutzfeldt-Jakob disease in 2, transient neurological deficit in 2, unilateral metastatic brain tumor in 1, corticobasal degeneration in 1, multisystem atrophy in 1, progressive supranuclear palsy in 1, meningoencephalitis in 1, gait disturbance in 1, and headache in 1. All these disorders

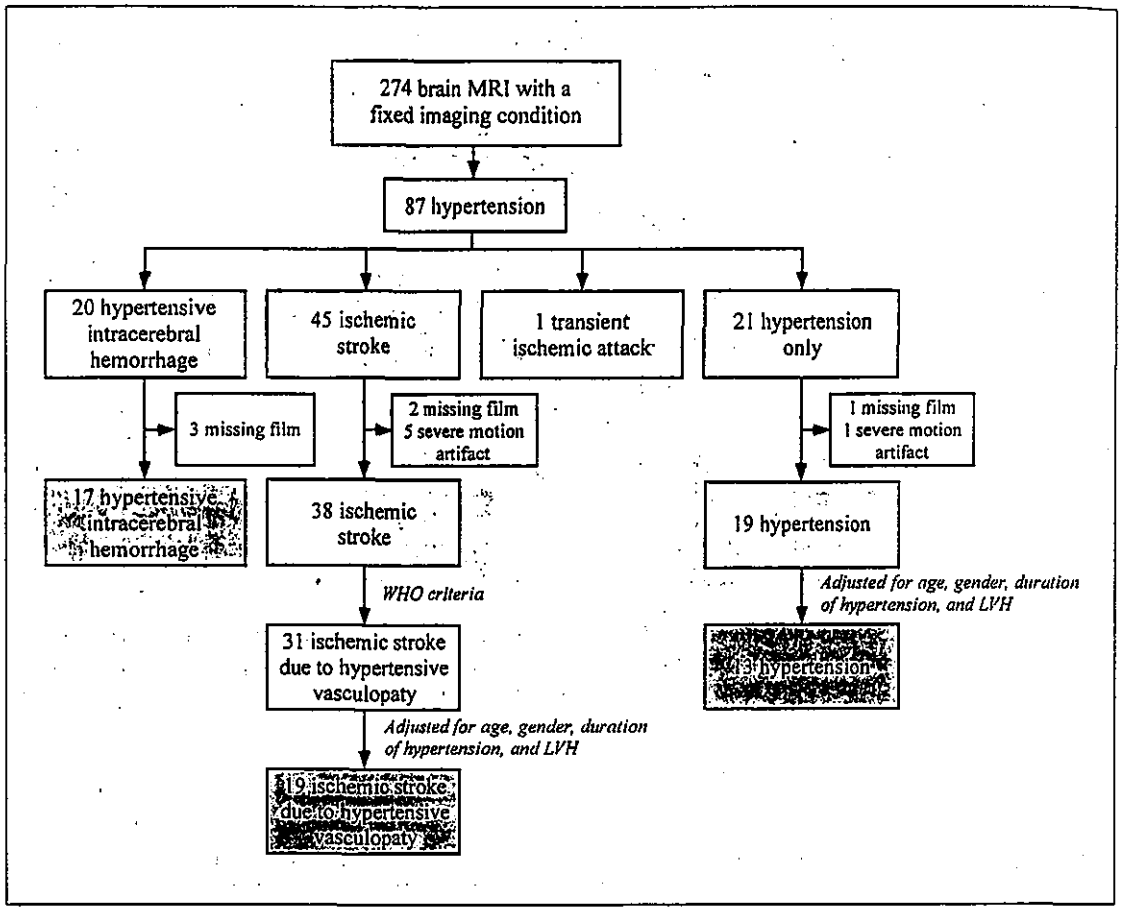

Fig. 2. Flow chart of subject selection. LVH stands for left ventricular hypertrophy.

were considered, at least at the time of the MRI examination, to have no influence on T_2-weighted MRI signals, in the cerebral hemisphere evaluated.

Definition of Vascular Risk Factors and MRI Condition

According to the JNC-7 report [11], hypertension was defined as a systolic blood pressure of ≥140 mm Hg or a diastolic blood pressure of ≥90 mm Hg based on the mean of 2 or more properly measured supine or seated blood pressure readings on 2 or more occasions. Upon reviewing clinical charts, both systolic and diastolic blood pressures were calculated as a mean value of at least 2 measurements on the day of the MRI examination for inpatients and during the last 2 weeks for outpatients. Diabetes mellitus was defined as having both symptoms of diabetes and a casual plasma glucose level of >11.1 mmol/l, a fasting plasma glucose level of >7.0 mmol/l, or a 2-hour plasma glucose level of >11.1 mmol/l during an oral glucose tolerance test [12]. Hypercholesterolemia was defined as >240 mg/dl total serum cholesterol and hypertriglycerolemia as >150 mg/dl fasting serum triglycerides. Smoking meant a history of smoking. Left ventricular hypertrophy was defined as either a cardiothoracic ratio of ≥50% on a chest X-ray, SV1 or SV2 + RV5 >3.5 mV on an electrocardiogram, or a posterior wall thickness of >12 mm on an echocardiogram. Serum creatinine was assessed in cases without nephropathy considered to result from nonhypertensive causes. All assessments were performed on each of the 49 patients.

MRI was performed using a 1.5-tesla superconductive scanner (Siemens Magnetom Vision, Erlangen, Germany) with a circular polarized head coil. Turbo spin-echo pulse sequences were routinely used to generate both T_1-weighted (a repetition time of 670.0 ms, an echo time of 14.0 ms) and T_2-weighted (a repetition time of 4,500.0 ms and an echo time of 96.0 ms) axial images of the brain. In each, the number of excitations was 2 and the scanning duration was 258 s. A matrix of 196 × 512 (phase × frequency) and a field of view of 173 × 230 mm were used. The slices were 5.0 mm thick and separated by a 1.0-mm interscan gap. Image reconstruction was carried out using two-dimensional Fourier transformation; smoothing was not performed.

Reliability Test and MRI Evaluation

To test the reliability of the manual measurement of LHOs, we prepared a card with a half-cut-out image of each patient at the uppermost corona radiata slice. We selected the side with fewer organic lesions (fig. 3). This way, any patient's identifying information including date, name, age as well as main organic lesion, was

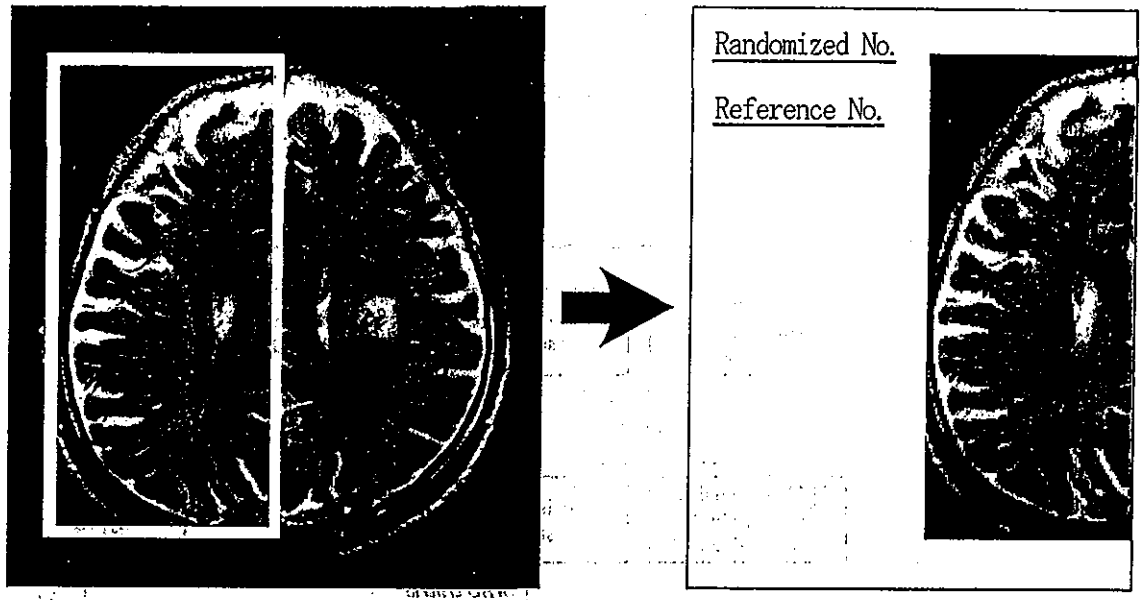

Fig. 3. Schema of the half-cut-out image card for the reliability test. We selected the uppermost corona radiata slice of the T_2-weighted MRI for each patient (left), and cut out the hemisphere with fewer organic lesions (right) in order to remove bias such as patient identifying information.

masked in the reliability test. Each card was presented to raters at random. At a point of two-thirds the distance from the lateral convexity to the lateral wall of the lateral ventricle, as we previously reported [5], we determined whether LHOs were present or absent, counted the number of LHOs with the naked eye, and measured the diameter of the LHOs with a 15× scale loupe. When a rater judged that the margin of the LHO diameter was unclear, it was excluded from the scaling. Therefore, in nearly all cases, the number of LHOs counted using a loupe was less than that of LHOs counted with the naked eye. The mean (SD) anterioposterior diameter ratio of the lateral ventricle to the brain on the slice studied was 0.35 (0.08) in the HIH, 0.33 (0.09) in the group with ischemic stroke due to hypertensive vasculopathy, and 0.32 (0.06) in hypertension-only group. Therefore, the measurement area of ischemic stroke due to hypertensive vasculopathy and hypertension-only was extended to a ratio of 0.35 (0.08) and 0.35 (0.04), respectively, to adjust to the HIH group. The measurement was taken from the right side in 10 patients, and from the left side in 7 patients in the HIH group, in 13 from the right side and in 6 from the left side in the group with ischemic stroke due to hypertensive vasculopathy, and in 6 from the right side and in 7 from the left side in the hypertension group. Using the half-cut-out images, an interrater test was performed by 4 independent raters (2 neurologists M.H. and S.T., and 2 stroke specialists K.M. and H.O.). An intrarater test was performed: one of the authors (M.H.) repeated the measurements four times in a 2-week period. Finally, presence of LHO, mean number, and mean diameter including the number of measurements were averaged by 4 raters for each patient.

Concerning other MRI findings, periventricular hyperintensities and subcortical white matter hyperintensities were scored as well based on Scheltens' scale (0–6 and 0–24, respectively) [13]. Microbleeds were defined on T_2-weighted MRIs as homogeneous, round, hypointense areas between 2 and 5 mm in diameter. Hypointense areas considered to be calcifications at the globus pallidus or a blood vessel were not regarded as microbleeds. Lacunar infarcts were defined as small, round, or oval-shaped lesions (<1.5 cm greatest diameter) [11], with both hypointensity on T_1-weighted and hyperintensity on a T_2-weighted MRI. The numbers of lacunar infarcts and microbleeds were counted on each slice. All MRI results were reviewed by an author (M.H.).

Statistical Methods

We compared the HIH group, the group with ischemic stroke due to hypertensive vasculopathy and the hypertension-only group using the Kruskal-Wallis H test with Scheffé's post hoc procedure, the Mann-Whitney U test, or the χ^2 test. Polynominal logistic regression analysis was performed to verify the independent explanatory variables for HIH by including all variables with $p < 0.05$ in the group comparison (current smoker, serum creatinine, antithrombotic medication, number of lacunar infarcts, microbleeds, and number and diameters of LHOs). Interrater and intrarater reliability was determined by calculating an intraclass correlation coefficient (ICC) through analysis of variance. This coefficient can be interpreted as a κ statistic: an ICC of 1.0 suggests perfect reliability, and an ICC >0.75 is generally considered to represent excellent reliability [14]. Finally, we evaluated each LHO number and diameter in relation to various clinical and radiological background factors using the Mann-Whitney U test or Spearman's rank test. Statistical significance was set at $p = 0.05$.

Table 1. Patients' characteristics

	HIH (n = 17)	Ischemic stroke due to hypertensive vasculopathy (n = 19)	Hypertension only (n = 13)	p value
Mean age, years (SD)	64.8 (10.3)	65.3 (6.7)	64.4 (10.8)	0.938
Male, n (%)	12 (70.6)	14 (73.7)	9 (69.2)	0.905
Mean systolic blood pressure, mm Hg (SD)	137 (15)	147 (20)	133 (12)	0.086
Mean diastolic blood pressure, mm Hg (SD)	86 (12)	85 (12)	82 (7)	0.394
Mean duration of hypertension, years (SD)	14.6 (9.1)	16.5 (8.5)	15.6 (11.3)	0.752
Antihypertensive medication, n (%)	13 (76.5)	12 (63.2)	3 (30.8)	0.062
Calcium channel blocker, n (%)	7 (41.1)	9 (47.4)	3 (23.1)	0.371
ACE inhibitor, n (%)	1 (5.9)	1 (5.3)	1 (7.7)	0.960
Controlled, n (%)	6 (35.3)	4 (21.1)	8 (61.5)	0.065
Diabetes mellitus, n (%)	6 (35.3)	12 (57.1)	2 (15.4)	0.059
Antidiabetic medication, n (%)	4 (23.5)	6 (31.5)	1 (7.7)	0.865
Controlled, n (% of diabetes)	3 (50.0)	3 (25.0)	1 (50.0)	0.598
Hypercholesterolemia, n (%)	6 (35.3)	6 (31.6)	4 (30.8)	0.959
Hypertriglycerolemia, n (%)	2 (11.8)	4 (21.1)	2 (15.3)	0.678
Statins, n (% of hyperlipidemia)	4 (66.7)	9 (100)	3 (75.0)	0.781
Controlled, n (% of hyperlipidemia)	3 (50.0)	3 (33.3)	3 (17.6)	0.234
Smoking, n (%)	9 (52.9)	10 (52.6)	5 (29.4)	0.919
Current smoker, n (%)	0 (0)	3 (15.8)	3 (17.6)	0.003
Left ventricular hypertrophy, n (%)	13 (76.5)	15 (79.0)	10 (76.9)	0.982
Serum creatinine, mg/dl (SD)	0.99 (0.25)	1.21 (0.43)[1]	0.89 (0.23)	0.044
Antithrombotic medication, n (%)	1 (5.9)	8 (42.1)	1 (7.7)	0.011

ACE = Angiotensin-converting enzyme.
[1] One case of glomerulonephritis was excluded.

Results

Clinical Background, Reliability Test, and MRI Results

Among the groups with HIH, ischemic stroke due to hypertensive vasculopathy and hypertension only, significant differences were found in current smokers ($p = 0.003$), patients with serum creatinine levels ($p = 0.044$), and patients on antithrombotic medication ($p = 0.011$) (table 1). Post hoc analysis showed significant differences in current smokers (HIH vs. ischemic stroke due to hypertensive vasculopathy; $p < 0.001$, and vs. hypertension only; $p = 0.001$) and the use of antithrombotic medication (HIH vs. ischemic stroke due to hypertensive vasculopathy; $p = 0.012$). No significant difference was found in serum creatinine by multiple comparisons.

Excellent reliability was identified for LHO numbers on interrater test ($\kappa = 0.92$, 95% CI = 0.88–0.95, SEM = 2.0%) and intrarater test ($\kappa = 0.95$, 95% CI = 0.92–0.97, SEM = 4.8%), LHO diameter on intrarater test ($\kappa = 0.89$, 95% CI = 0.83–0.93, SEM = 7.1%), and measured number of LHO diameters on interrater test ($\kappa = 0.87$, 95% CI = 0.80–0.92, SEM = 6.5%) and intrarater test ($\kappa = 0.94$, 95% CI = 0.90–0.96, SEM = 8.4%) tests. Interrater reliability of LHO diameters was low ($\kappa = 0.57$, 95% CI = 0.33–0.74, SEM = 33.1%).

In MRI results (table 2), no significant difference was found among the three groups in mean time from onset to MRI, severity of periventricular hyperintensities and subcortical white matter hyperintensities, and presence of LHO. There were significant differences in mean lacunar infarct number ($p < 0.0001$), in both the presence and number of microbleeds ($p = 0.015$ and 0.008, respectively), and the number of microbleeds at the basal ganglia or thalamus ($p = 0.007$). Post hoc analysis showed that compared with the hypertension-only group, the HIH group and the group with ischemic stroke due to hypertensive vasculopathy had a significantly higher mean lacunar infarct number ($p = 0.005$ and 0.001, respectively) and a higher presence of microbleeds ($p = 0.010$ and 0.012,

Table 2. MRI results

	HIH (n = 17)	Ischemic stroke due to hypertensive vasculopathy (n = 19)	Hypertension only (n = 13)	p value
Mean time from onset to MRI, days (range)	336.3 (12–1184)	203.8 (4–1417)		0.361*
Mean lacunar infarct number (range)	3.3 (0–12)	3.6 (0–10)	0	<0.0001
Periventricular hyperintensities				
Mean Scheltens's score (range)	1.7 (0–6)	1.7 (0–6)	1.2 (0–6)	0.556
Subcortical white matter hyperintensities				
Mean Scheltens's score (range)	9.7 (0–24)	9.1 (0–24)	5.0 (0–24)	0.769
Microbleeds				
Presence, %	70.6	68.4	23.1	0.015
Mean number (range)	3.0 (0–11)	2.9 (0–20)	0.4 (0–2)	0.008
Distribution, n (range)				
Lobar region	0.4 (0–2)	0.3 (0–4)	0.1 (0–1)	0.487
Basal ganglia/thalamus	2.0 (0–6)	1.8 (0–11)	0.2 (0–2)	0.007
Infratentorial	0.9 (0–4)	0.6 (0–5)	0.1 (0–1)	0.289
LHO				
Presence, %	100	100	100	
Mean number (SD)	10.7 (4.5)	7.0 (3.6)	5.5 (2.1)	0.002
Mean diameter, mm (SD)	0.67 (0.14)	0.59 (0.09)	0.56 (0.06)	0.007
Mean measured number (SD)	7.1 (3.0)	4.5 (2.2)	3.7 (1.1)	0.001

* Mann-Whitney U test between HIH and ischemic stroke due to hypertensive vasculopathy groups, excluding 2 cases of unclear onset in the ischemic stroke due to hypertensive vasculopathy.

respectively). No significant difference was found by multiple comparison in the total number of microbleeds and the number of microbleeds at the basal ganglia or thalamus. Concerning LHOs, there were significant differences in number, diameter, and measured number among the three groups (p = 0.002, 0.007, and 0.001, respectively). Post hoc analysis showed a significant difference in LHO number (HIH vs. ischemic stroke due to hypertensive vasculopathy; p = 0.013, and vs. hypertension only; p = 0.001, respectively), LHO diameter (HIH vs. hypertension only; p = 0.015), and the measured LHO number (HIH vs. ischemic stroke due to hypertensive vasculopathy; p = 0.008, and vs. hypertension only; p = 0.001, respectively).

Indicator of HIH and the Relationship to Each Background Factor

In polynomial logistic regression analysis, the LHO number was the most significant independent indicator of HIH (OR = 1.90, 95% CI = 1.16–3.12, p = 0.011) and the number of microbleeds was significant as well (OR 3.73, 95% CI = 1.10–12.65, p = 0.034). When LHO diameter was included in this model, it was the most significant independent indicator of HIH (OR per 0.1 mm: 1.76, 95% CI = 1.16–3.12, p = 0.011) followed by the number of microbleeds (OR = 2.90, 95% CI = 1.13–7.48, p = 0.028).

In categorical clinical or radiological variables, the LHO numbers of patients that took antihypertensive medication were significantly higher (p = 0.047) and the LHO diameters of current smokers were significantly lower (p = 0.016). No other significant difference was found. In continuous clinical or radiological variables, the LHO number and diameter did not significantly correlate with the time from onset to MRI in the HIH group (r = –0.24; p = 0.342, and r = 0.04; p = 0.868, respectively) and in the group with ischemic stroke due to hypertensive vasculopathy (r = 0.15; p = 0.523, and r = –0.05; p = 0.826, respectively), or with the size of hematomas (r = 0.18; p = 0.510, and r = 0.31; p = 0.263, respectively; n = 15). Diastolic blood pressure showed a significant correlation with LHO number among all patients (r = 0.29, p = 0.047). Other continuous values, including the degree of periventricular hyperintensities and subcortical white matter hyperintensities, lacunar infarct number, or number of microbleeds did not relate to either to LHO number or to diameter.

Discussion

This study showed with high reliability that LHO number is closely linked to the occurrence of HIH. Since linear hyperintensities in cerebral white matter on T_2-weighted MRIs are related to age [15–17] and severity of hypertension [5], we carefully adjusted age, gender, duration of hypertension and left ventricular hypertrophy of the group with ischemic stroke due to hypertensive vasculopathy and the hypertension-only group to those of the HIH group. We could not balance the serum creatinine level among groups due to the limited number of patients. The result was that the serum creatinine level tended to be higher in the group with ischemic stroke due to hypertensive vasculopathy. Although there was no a significant difference by multiple comparison, this fact is considered to reflect a common pathological basis in both lacunar infarct (the number of which was also higher in this group) and elevated serum creatinine [18]. In general, since both HIH and ischemic stroke due to hypertensive vasculopathy belong to stage III of the hypertension severity of WHO classification [8], it can be said that our study investigated the details of stage III.

Since HIH and lacunar infarction may share a common or related casual vessel lesion such as lipohyalinosis, depicted, in part, as microbleeds on T_2(*)-weighted MRIs [1, 19], it is difficult to predict whether HIH or ischemic stroke will occur in the most hypertensive patients, based on microbleeds. On the other hand, linear hyperintensities in the cerebral white matter on T_2-weighted MRIs were previously paid little attention from a clinical viewpoint. We have recently reported that *LHOs increase* in diameter according to the severity of hypertensive organopathy, and that they are probably based on arteriolar tortuosity of the white matter medullary artery and its dilated perivascular spaces [5, 6]. Overall, based on the results of our studies, prominent LHOs may reflect advanced microangiopathy, such as arteriolar tortuosity of the perforating arteries as well as that of the white matter medullary artery with an increased risk for bleeding, although we could not assess the former arterial territory using coronal or sagittal MRIs.

The actual source of bleeding in HIH has never been proven except for microaneurysm [20], but some reports have suggested the possibility of other mechanisms of vascular rupture in HIH [19, 21, 22]. Interestingly, Challa et al. [21] identified tortuous arterioles of the perforating arteries with an increased susceptibility of vascular rupture in HIH brains using high-resolution microangiography. Based on this report, while the relationship between white matter medullary arteries and perforating arteries remains *unproven*, prominent LHOs may reflect the cause of bleeding through a mechanism such as microdissection [21].

On the other hand, it may be said that, theoretically, LHOs reflect the epiphenomenon of HIH. From a pathological viewpoint, it has never been reported that hemorrhage affects the contralesional hemisphere except for mass effect. From the viewpoint of cerebral blood flow, it was shown that the autoregulation of global cerebral blood flow is constant in acute and treated stages [23]. Hyperperfused areas are sometimes found in the ipsilesional hemisphere in acute intracerebral hemorrhage, but never in the contralesional hemisphere [24–26]. From a clinical viewpoint, our results did not show any temporal relationship between the time from onset to MRI and LHOs, although the number of patients was small. Overall, it seems difficult to say that prominent LHOs *result from* intracerebral hemorrhage.

The interrater test of LHO diameter unexpectedly showed low reliability. This is likely due to the limitations of MRI resolution in manual measurements with a loupe. A smaller diameter of, at least, the y-axis direction of a pixel, a higher signal-to-noise ratio, and a shorter scan duration are imaging conditions to be considered for a future study, as well as an automatic measurement of LHOs such as segmentation. It has been unclear which factors related to high blood pressure cause LHOs, since the results of our study could not show any contributing factor. Since hemorrhagic stroke may occur in different types of blood pressure variation compared to ischemic stroke [27], it is considered that detailed clinical assessment including circadian blood pressure variation may be a key in unveiling the mechanism of LHO progression. Genetic susceptibility is also a considerable factor to be investigated [19]. Furthermore, the effect of antihypertensive treatment of LHOs has to be clarified in a long-term follow-up study.

In conclusion, the prominence of LHOs is closely linked to the occurrence of HIH. To clarify whether LHOs reflect the cause of and predict HIH, prospective, longitudinal studies are required using high-resolution MRI.

Acknowledgment

We thank Dr. A. Gregory Sorensen of the Department of Radiology, Massachusetts General Hospital NMR Center, for his valuable comments on this work.

References

1 Nighoghossian N, Hermier M, Adeleine P, Blanc-Lasserre K, Derex L, Honnorat J, Philippeau F, Dugor JF, Froment JC, Trouillas P: Old microbleeds are a potential risk factor for cerebral bleeding after ischemic stroke: A gradient-echo T2*-weighted brain MRI study. Stroke 2002;33:735–742.

2 Roob G, Lechner A, Schmidt R, Flooh E, Hartung HP, Fazekas F: Frequency and location of microbleeds in patients with primary intracerebral hemorrhage. Stroke 2000;31:2665–2669.

3 Kato H, Izumiyama M, Izumiyama K, Takahashi A, Itoyama Y: Silent cerebral microbleeds on T2*-weighted MRI: Correlation with stroke subtype, stroke recurrence, and leukoaraiosis. Stroke 2002;33:1536–1540.

4 Wong KS, Chan YL, Liu JY, Gao S, Lam WW: Asymptomatic microbleeds as a risk factor for aspirin-associated intracerebral hemorrhages. Neurology 2003;60:511–513.

5 Hiroki M, Miyashita K: Linear hyperintensity objects on magnetic resonance imaging related to hypertension. Cerebrovasc Dis 2001;11: 164–168.

6 Hiroki M, Miyashita K, Oda M: Tortuosity of the white matter medullary arterioles is related to the severity of hypertension. Cerebrovasc Dis 2002;13:242–250.

7 Gibby WA: MRI hardware, signal-to-noise ratio, and safety; in Zimmerman RA, Gibby WA, Carmody RF (eds): Neuroimaging: Clinical and Physical Principles. New York, Springer, 1997, pp 125–157.

8 WHO: Arterial hypertension. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1978;628:7–56.

9 Kothari RU, Brott T, Broderick JP, Barsan WG, Sauerbeck LR, Zuccarello M, Khoury J: The ABCs of measuring intracerebral hemorrhage volumes. Stroke 1996;27:1304–1305.

10 Special report from the National Institute of Neurological Disorders and Stroke. Classification of cerebrovascular diseases III. Stroke 1990;21:637–676.

11 Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee: The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 report. JAMA 2003;289:2560–2572.

12 WHO: Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications. Report of a WHO Consultation. 1. Diagnosis and Classification of Diabetes mellitus. Geneva, WHO, 1999.

13 Scheltens P, Barkhof F, Leys D, Pruvo JP, Nauta JJ, Vermersch P, Steinling M, Valk J: A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. J Neurol Sci 1993;114:7–12.

14 Armitage P, Berry G, Matthews JN: Statistical methods in medical research. Oxford, Blackwell Science, 2002, pp 698–707.

15 Heier LA, Bauer CJ, Schwartz L, Zimmerman RD, Morgello S, Deck MD: Large Virchow-Robin spaces: MR clinical correlation. AJNR Am J Neuroradiol 1989;10:929–936.

16 Munoz DG, Hastak SM, Harper B, Lee D, Hachinski VC: Pathologic correlates of increased signals of the centrum ovale on magnetic resonance imaging. Arch Neurol 1993;50: 492–497.

17 Braffman BH: The aging brain and neurodegenerative disorders; in Zimmerman RA, Gibby WA, Carmody RF (eds): Neuroimaging. Clinical and Physical Principles. New York, Springer, 1997, pp 951–978.

18 Longstreth WT Jr, Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR: Lacunar infarcts defined by magnetic resonance imaging of 3,660 elderly people: The Cardiovascular Health Study. Arch Neurol 1998;55:1217–1225.

19 Lammie GA: Hypertensive cerebral small vessel disease and stroke. Brain Pathol 2002;12: 358–370.

20 Fisher CM: Hypertensive cerebral hemorrhage. Demonstration of the source of bleeding. J Neuropathol Exp Neurol 2003;62:104–107.

21 Challa VR, Moody DM, Bell MA: The Charcot-Bouchard aneurysm controversy: Impact of a new histologic technique. J Neuropathol Exp Neurol 1992;51:264–271.

22 Anim JT, Kofi AD: Hypertension, cerebral vascular changes and stroke in Ghana. 1. Microaneurysm formation and stroke. J Pathol 1984; 143:177–182.

23 Powers WJ, Zazulia AR, Videen TO, Adams RE, Yundt KD, Aiyagari V, Grubb RL Jr, Diringer MN: Autoregulation of cerebral blood flow surrounding acute (6 to 22 hours) intracerebral hemorrhage. Neurology 2001;57:18–24.

24 Rosand J, Eskey C, Chang Y, Gonzalez RG, Greenberg SM, Koroshetz WJ: Dynamic single-section CT demonstrates reduced cerebral blood flow in acute intracerebral hemorrhage. Cerebrovasc Dis 2002;14:214–120.

25 Mayer SA, Lignelli A, Fink ME, Kessler DB, Thomas CE, Swarup R, Van Heertum RL: Perilesional blood flow and edema formation in acute intracerebral hemorrhage: A SPECT study. Stroke 1998;29:1791–1798.

26 Miyazawa N, Mitsuka S, Asahara T, Uchida M, Fukamachi A, Fukasawa I, Sasaki H, Nukui H: Clinical features of relative focal hyperfusion in patients with intracerebral hemorrhage detected by contrast-enhanced xenon CT. AJNR Am J Neuroradiol 1998;19:1741–1746.

27 Kario K, Pickering TG, Matsuo T, Hoshide S, Schwartz JE, Shimada K: Stroke prognosis and abnormal nocturnal blood pressure falls in older hypertensives. Hypertension 2001;38:852–857.

ELSEVIER

Atherosclerosis 176 (2004) 355–360

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Potential of free-form TFPI and PAI-1 to be useful markers of early atherosclerosis in a Japanese general population (the Suita Study): association with the intimal-medial thickness of carotid arteries

Toshiyuki Sakata [a,*], Toshifumi Mannami [b,1], Shunroku Baba [b,2], Yoshihiro Kokubo [b], Kazuomi Kario [c], Akira Okamoto [a], Kousuke Kumeda [d], Naoki Ohkura [e,3], Yoshiaki Katayama [a], Toshiyuki Miyata [e], Hitonobu Tomoike [b], Hisao Kato [e]

[a] *Laboratory of Clinical Chemistry, National Cardiovascular Center, Fujishirodai 5-7-1, Suita, Osaka 565-8565, Japan*
[b] *Department of Preventive Cardiology, National Cardiovascular Center, Fujishirodai 5-7-1, Suita, Osaka 565-8565, Japan*
[c] *Department of Cardiology, Jichi Medical School, Tochigi, Japan*
[d] *Chemo-Sero Therapeutic Research Institute, Kumamoto, Japan*
[e] *Research Institute, National Cardiovascular Center, Fujishirodai 5-7-1, Suita, Osaka 565-8565, Japan*

Received 7 May 2003; received in revised form 29 April 2004; accepted 4 May 2004
Available online 14 July 2004

Abstract

This study assessed markers of vascular endothelial cell dysfunction associated with early atherosclerosis in carotid arteries. We measured the plasma levels of free-form tissue factor pathway inhibitor (free TFPI), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) in 522 adults without cardiovascular disease enrolled in the Suita Study. For each sex, we analyzed the association of the degree of intimal-medial thickness (IMT) with hemostatic markers using logistic regression analysis considering potential confounding risk factors, including age, body mass index, lifestyle (current smoking and drinking), illness (diabetes mellitus and hyperlipidemia), systolic blood pressure, and antihypertensive drug use. The age-adjusted levels of free TFPI and PAI-1 were positively and independently associated with the degree of IMT for men. Even after adjustment for all confounding factors, the level of PAI-1 was positively associated with the degree of IMT. These results indicate that measurement of the levels of free TFPI and PAI-1 is a potentially useful tool for the detection of early atherosclerosis in men.

Keywords: Atherosclerosis; Endothelium-derived factors; Hypertension

1. Introduction

Measurement of the intimal-medial thickness (IMT) of carotid arteries has been used as a non-invasive endpoint in epidemiological studies and clinical trials to assess the progression and regression of atherosclerosis [1,2]. Furthermore, IMT has recently been used not only as a surrogate endpoint for atherosclerosis of the coronary artery but also as a good indicator of the presence and extent of coronary artery disease [3–6]. Case-reference studies in a general population have been performed in regard to the association between markers of vascular endothelial cell dysfunction and atherosclerosis by measuring IMT of the carotid artery [7–9]. However, to detect early atherosclerosis, it is essential to study the association between these markers and the extent of atherosclerosis, using a general population free from cardiovascular disease (CVD).

* Corresponding author. Tel.: +81 6 6833 5012x2296; fax: +81 6 6835 1176.
E-mail address: tsakata@hsp.ncvc.go.jp (T. Sakata).
[1] Present address: Department of Hygiene/Public Health, Faculty of Medicine, Kagawa University, Kagawa 761-0701, Japan.
[2] Present address: The Second Hanwa Hospital, 3176 Fukaikitamachi, Sakai-City, Osaka 599-8271, Japan.
[3] Clinical Molecular Biology, Faculty of Pharmaceutical Science Teikyo University, 1091-1 Suarashi, Sagamiko, Tsukui-gun, Kanagawa 199-0195, Japan.

0021-9150/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2004.05.017

In this study, we focused on the association between three markers of endothelial cell dysfunction, namely free-form tissue factor pathway inhibitor (free TFPI), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) and IMT of carotid arteries in a Japanese general population (the Suita Study). Plasma concentrations of vWF and PAI-1 have previously been used as surrogate markers of endothelial damage [10,11]. TFPI inhibits tissue factor-initiated coagulation by binding to factor Xa and tissue factor-activated factor VII complex [12,13]. Most TFPI is synthesized by vascular endothelial cells, and is distributed into at least four pools in vivo. The majority of TFPI synthesized by vascular endothelial cells is associated with endothelial cells, whereas other pools circulate in the blood as complexes with lipoproteins (Lp-TFPI) or as a free form (free TFPI). A minor pool of TFPI is present in platelets. It has been demonstrated that free TFPI strongly correlates with endothelial cell markers such as thrombomodulin, vWF, and tissue-type plasminogen activator, whereas total TFPI does not [14]. There is a strong, positive correlation between the free TFPI and endothelial cell-associated TFPI levels [15]. Therefore, we selected free TFPI as a marker of endothelial cell dysfunction, instead of Lp-TFPI or total TFPI.

Here, we have demonstrated the potential of free TFPI and PAI-1 to be useful markers of early atherosclerosis by studying their association with IMT in relation to conventional risk factors for CVD.

2. Methods

2.1. Study population

The study population was based on samples randomly selected from the residents of Suita, a city located in the second largest urban area in Japan (the Suita Study) [5]. The subjects have been visiting the National Cardiovascular Center every 2 years since 1989 for regular health checkups. Only subjects who provided written informed consent to have a blood examination were enrolled in this study. The subjects included 245 men and 277 women who were free of cardiovascular disease, aged from 34 to 91 years, and attended the National Cardiovascular Center from 5 August 1998 to 24 December 1998. Subjects were classified as smokers if they smoked at least one cigarette per day. Subjects were defined as hypertensive if their diastolic blood pressure was ≥95 mmHg, their systolic blood pressure was ≥160 mmHg, or they were taking antihypertensive medication. Subjects whose fasting blood glucose levels were ≥7.78 mmol/L, whose blood glucose levels were ≥11.11 mmol/L 2 h after a 75-g oral glucose loading, or who were taking antidiabetic medication were defined as diabetic. Subjects whose total serum cholesterol level was ≥5.68 nmol/L (220 mg/dl), or who were taking anti-hypercholesterolemic medication were defined as having hypercholesterolemia.

2.2. IMT measurements

The details of the ultrasonic carotid examination have previously been published [16]. We used a high-resolution B-mode ultrasonic machine with 7.5-MHz transducers, yielding an axial resolution of 0.2 mm. The regions between 30 mm proximal from the beginning of the dilation of the bifurcation bulb and 15 mm distal from the flow divider of both common carotid arteries were scanned. All measurements were made at the time of scanning using the instrument's electronic caliper and were recorded as photocopies. The IMT in common carotid arteries was measured on a longitudinal scan of the common carotid arteries at a point 10 mm proximal from the beginning of the dilation of the bifurcation bulb. We defined the IMT as mean IMT of the near and far walls at the point of measurement.

2.3. Blood collection and analysis

After a minimum 12-h fast and between 10 a.m. and 1 p.m., blood samples for hemostatic profile were collected into disposable, siliconized, evacuated glass tubes containing 0.1 vol. of 3.13% trisodium citrate, and blood collected in a second tube was used for the coagulation assay. The samples were centrifuged at 4600 × g for 10 min at room temperature within 1 h of collection. The PAI-1 antigen level was immediately determined, and the remaining plasma was aliquoted in plastic tubes and stored at −80 °C until use. The thawed samples were used to measure free TFPI and vWF.

The antigen level of free TFPI was measured by a sandwich enzyme immunoassay method [17]. The coefficient of intra-assay variation for the assay was 2.7%. The antigen levels of PAI-1 and vWF were automatically measured by latex photometric immunoassay using an LPIA-tPAI kit (Mitsubishi Kagaku Medical) and STA liatest vWF kit (Diagnostica Stago), respectively. The coefficients of intra-assay variation of PAI-1 and vWF were 1.0 and 4.3%, respectively.

2.4. Statistical analysis

All statistical analyses were performed independently by sex. We first used Spearman correlation analysis to assess the association between the progression of IMT and the analyzed parameters (Tables 1 and 2). We then used ANCOVA to investigate whether plasma levels of free TFPI, PAI-1, and vWF are positively and independently associated with the degree of carotid intimal thickness or not (Table 3). We have performed two types of adjustments. First, adjustments were made for age only. Second, further adjustments were made for lifestyle (drinking and smoking), illness (diabetes, hypercholesterolemia), body mass index, systolic blood pressure, and antihypertensive drug use. Differences with a value of $P < 0.05$ for ANCOVA were

Table 1
Demographic characteristics and unadjusted hemostatic parameters according to rank of intimal-medial thickness (IMT) of the carotid artery in men

	IMT-rank				P
	Q1 ($n = 58$)	Q2 ($n = 70$)	Q3 ($n = 57$)	Q4 ($n = 60$)	
Median of IMT (mm)	0.73	0.83	0.93	1.05	
Age (year)	47.4 ± 7.8	59.2 ± 9.2	69.2 ± 8.7	70.4 ± 8.0	<0.0001
Current drinking (%)	79	73	67	62	<0.0009
Smoker (%)	78	73	67	62	<0.0007
Body mass index (kg/m^2)	23.2 ± 3.3	23.7 ± 3.2	23.4 ± 3.3	22.8 ± 3.1	<0.4217
Diabetes (%)	0	3	5	10	<0.0511
Hypertension (%)	7	29	32	45	<0.0001
Hypercholesterolemia (%)	7	11	9	15	<0.1685
LDL-cholesterol (mg/dl)	113.9 ± 27.5	124.8 ± 29.5	120.3 ± 26.7	131.0 ± 26.4	<0.0060
HDL-cholesterol (mg/dl)	60.8 ± 17.2	56.1 ± 12.5	55.2 ± 16.1	55.2 ± 16.1	<0.0255
Free TFPI (ng/ml)	15.7 ± 4.5	16.0 ± 3.8	17.2 ± 3.2	18.2 ± 4.6	<0.0006
PAI-1 (ng/ml)	32.3 ± 25.2	32.9 ± 30.9	29.3 ± 19.7	33.0 ± 38.8	<0.2059
von Willebrand factor (%)	115.0 ± 43.7	137.1 ± 45.9	151.7 ± 54.9	160.8 ± 63.9	<0.0001

Values are mean ± S.D. or percent. *P*-values were calculated by simple linear regression analysis. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

Table 2
Demographic characteristics and unadjusted hemostatic parameters according to rank of intimal-medial thickness (IMT) of the carotid artery in women

	IMT-rank				P
	Q1 ($n = 66$)	Q2 ($n = 73$)	Q3 ($n = 63$)	Q4 ($n = 75$)	
Median of IMT (mm)	0.70	0.78	0.85	0.95	
Age (year)	45.8 ± 6.7	55.2 ± 7.6	62.2 ± 7.2	71.9 ± 7.8	<0.0001
Current drinking (%)	45	33	35	25	<0.0897
Smoker (%)	12	5	5	8	<0.6107
Body mass index (kg/m^2)	21.3 ± 2.5	21.6 ± 2.9	23.2 ± 3.5	22.9 ± 3.6	<0.0145
Diabetes (%)	2	0	5	4	<0.6649
Hypertension (%)	2	10	19	40	<0.0001
Hypercholesterolemia (%)	8	10	11	19	<0.0913
LDL-cholesterol (mg/dl)	115.4 ± 30.5	126.4 ± 30.0	137.9 ± 25.7	137.0 ± 26.5	<0.0001
HDL-cholesterol (mg/dl)	73.1 ± 17.9	69.2 ± 15.5	67.5 ± 16.7	62.1 ± 14.8	<0.0002
Free TFPI (ng/ml)	11.5 ± 3.2	14.9 ± 5.0	16.3 ± 4.2	17.5 ± 4.8	<0.0001
PAI-1 (ng/ml)	20.2 ± 18.3	19.8 ± 12.8	25.7 ± 19.1	26.2 ± 18.0	<0.0707
von Willebrand factor (%)	107.8 ± 31.4	126.5 ± 41.8	136.5 ± 51.6	157.2 ± 59.2	<0.0001

Values are mean ± S.D. or percent. *P*-values were calculated by simple linear regression analysis. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

considered to be significant. All analyses were performed with SAS statistical software (release 8.2 SAS Institute Inc).

3. Results

3.1. Demographic characteristics and unadjusted parameters according to rank of IMT of carotid arteries

We measured IMT in a general population, divided it into four quartiles by sex, and analyzed the demographic characteristics and unadjusted parameters according to IMT rank, as shown in Tables 1 and 2. The IMT median of each quartile (*Q*1, *Q*2, *Q*3 and *Q*4) is shown in the first column of each table. In both sexes, the plasma levels of free TFPI, vWF, and LDL-cholesterol as well as age and hypertension increased in a stepwise manner from the first to the forth IMT quartile.

3.2. Multivariate analysis of free TFPI, PAI-1, and vWF levels according to IMT rank

As summarized in Table 3, we analyzed the plasma levels of free TFPI, PAI-1, and vWF according to IMT ranks after either adjusting for age only or adjusting for age, lifestyle (drinking and smoking), body mass index, systolic blood pressure, diabetes, hypercholesterolemia, and hypertensive drug use. Age adjusted free TFPI levels in men increased in a stepwise manner from the first to the fourth IMT quartile ($P = 0.003$, for trend) and the levels of free TFPI in the third and the fourth quartiles compared to the lowest IMT quartile remained statistically significant in the multivariate analysis. However, the statistically significant increases of free TFPI