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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Sex-Related Differences in the Risk Factor Profile and Medications of Patients With Atrial Fibrillation Recruited in J-TRACE

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Background: Clinical characteristics, including risk factors for thromboembolism, and medications differ between men and women with atrial fibrillation (AF) in Western countries. Whether such a difference exists for Japanese patients with AF is unclear, so data from J-TRACE were used to investigate this issue.

Methods and Results: A total of 2,892 patients (2,028 men, 864 women; 70.3 years old) with AF were analyzed for the respective prevalences of risk factors and medications. CHADS2 score was calculated to determine thromboembolic risk level. Women were older (P<0.001), and more frequently had heart failure (P<0.001), and hypertension (P=0.051) than men. The proportion of subjects aged 75 years or older was higher among women than among men (P<0.001). CHADS2 score was therefore significantly higher in women than in men (2.05±1.29 vs 1.88±1.33, P<0.001). Sex-related differences were not observed for the prevalence of diabetes mellitus, myocardial infarction or ischemic stroke, nor did warfarin usage differ between men and women.

Conclusions: Sex-related differences were observed in the risk factor profile and medications of Japanese patients with AF. CHADS2 score was higher in women than in men. (*Circ J* 2010; **74:** 650–654)

Key Words: Atrial fibrillation; CHADS2 score; Clinical characteristics; Medications; Sex differences

Atrial fibrillation (AF) is a common cardiac arrhythmia seen in general practice as well as in the cardiology clinic. The prevalence of AF differs between men and women in Western countries,[1–3] and also in Japan.[4,5] Several studies have reported that there are sex-related differences in the clinical characteristics and medications of patients with AF.[6–10] A prospective, cohort study indicated that the effects of AF on the risk of stroke were greater in women than in men after adjustment for age and comorbidity.[9] Other studies also showed that AF is associated with an increase in cardiovascular events, including mortality and stroke, especially in women.[7,11,12] Some risk stratification schemes consider women to be at high risk for ischemic stroke,[13,14] while others do not.[15,16] However, because the sex-related differences in risk factors for cardiovascular diseases and medications of Japanese patients with AF have yet to be clarified, registry data for a large, nation-wide, multicenter, cooperative study, J-TRACE (The Japan Thrombosis Registry for Atrial Fibrillation, Coronary or Cerebrovascular Events),[17,18] were analyzed to address this issue in the present study.

Methods

The details of J-TRACE have been reported elsewhere.[17,18] Briefly, J-TRACE has a steering committee of 5 members and 41 regional coordinators selected from 10 regions of Japan (Appendix 1). Recruitment of patients to investigate risk factor profiles and current status of medications for risk factors and for prevention of cardiovascular events in patients with

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Table 1. Clinical Characteristics of Japanese Patients With AF

	Men (n=2,028)	Women (n=864)	P value
Age (years)	69.4±9.4	72.6±8.5	<0.001
≥75 years (%)	32.0	44.5	<0.001
Chronic AF* (%)	68.8 (1,062/1,543)	66.1 (462/699)	0.199
BMI (kg/m²)	23.8±3.2	23.4±4.1	<0.001
CHF (%)	17.0	27.1	<0.001
Hypertension (%)	57.2	61.1	0.051
DM (%)	19.1	16.7	0.125
Ischemic stroke (%)	29.4	26.3	0.089
VHD (%)	10.1	21.1	<0.001
MI (%)	7.6	5.9	0.096
HC (%)	25.1	35.5	<0.001
Drinker (%)	46.3	5.2	<0.001
Smoker (%)	21.2	4.3	<0.001
CHADS2 score	1.88±1.33	2.05±1.29	<0.001

Data are mean±SD or % of patients.
*In the myocardial infarction and stroke categories; subtypes of AF were not specifically determined.
AF, atrial fibrillation; BMI, body mass index; CHF, congestive heart failure; DM, diabetes mellitus; MI, myocardial infarction; VHD, valvular heart diseases including valve replacement; HC, hypercholesterolemia.

prior stroke, myocardial infarction (MI) or AF began in January 2005 and ceased in December 2006.

Study Population

Patients aged 20–90 years were eligible for enrollment if they had at least 1 of the 3 cardiovascular diseases (stroke, MI or AF). The study protocol was approved by an Institutional Review Board at each participating site and all patients gave informed consent. Those in the AF category, and those in the stroke and MI categories who also had AF, comprised the study subjects for this subanalysis of J-TRACE. Those in the recovery phase of acute MI or acute stroke were not eligible for enrollment in J-TRACE.

Baseline Characteristics

All subtypes of AF were included. AF was diagnosed electrocardiographically using standard diagnostic criteria. Risk factors and comorbidities were collected from the medical record as baseline data. Among them were hypertension, diabetes mellitus, hypercholesterolemia, valvular diseases, MI, ischemic stroke, congestive heart failure, smoking, and drinking. Regular use of medications, including anticoagulants, antiplatelet agents, and drugs for hypercholesterolemia, hypertension, and diabetes mellitus, was also determined from the medical record. Each patient's CHADS2 score[15] was calculated to determine the level of cardioembolic risk: 1 point was given for advanced age (≥75 years), hypertension, congestive heart failure, or diabetes mellitus, and 2 points for prior stroke or transient ischemic attack.

Statistical Analysis

Continuous variables are shown as the mean±SD, and categorical variables as percentages. Continuous variables were compared by analysis of variance or Student's t-test, and categorical variables with the chi-square test, with P<0.05 considered significant.

Table 2. Distribution of CHADS2 Scores

CHADS2 score	Men	Women
0	15.9	11.1
1	28.4	25.0
2	23.5	29.6
3	19.2	20.6
4	10.5	10.0
5	2.2	3.4
6	0.3	0.3

Figures are % of patients.
P<0.001 between men and women.

Table 3. Age and CHADS2 Score

	Age			P value
	<65 years	65–74 years	≥75 years	
Men	1.24±1.12 (n=572)	1.63±1.22 (n=808)	2.74±1.17 (n=648)	<0.001
Women	1.38±1.16 (n=153)	1.57±1.14 (n=326)	2.72±1.12 (n=385)	<0.001

Data are mean±SD.

Results

Risk Factor Profile

A total of 2,892 patients (2,028 men, 864 women; mean age, 70.3 years) with AF comprised the study group. Numbers of patients and their mean age in the 3 categories were as follows: AF category, 1,543 men (68.9±9.6 years old) and 699 women (72.4±8.5); stroke category, 399 men (70.6±8.4) and 141 women (73.0±8.3); MI category, 86 men (71.7±8.1) and 24 women (75.3±8.0). Their clinical characteristics are summarized in **Table 1**. Some of the characteristics exhibited differences by sex. Women were older (P<0.001), and more frequently had congestive heart failure (P<0.001), hypertension (P=0.051), valvular diseases or valve replacement (P<0.001), and hypercholesterolemia (P<0.001) than the men, but drank (P<0.001) and smoked (P<0.001) less frequently than men. The proportion of subjects aged 75 years or older was higher and body mass index was slightly but significantly lower in women than in men (P<0.001, each case). The prevalences of chronic AF, diabetes mellitus, MI, and ischemic stroke did not differ between men and women.

The CHADS2 score was slightly but significantly higher in women than in men (**Table 1**, P<0.001) because of their higher prevalence of older age (≥75 years), hypertension, and congestive heart failure. The distribution of CHADS2 scores differed significantly between men and women (**Table 2**, P<0.001). It increased with age for both men and women, but did not differ between men and women in any age group (**Table 3**).

Medications

Medications are summarized in **Table 4**. Use of warfarin and antiplatelet agents did not differ between men and women. Reflecting the differences in prevalence of hypertension and hypercholesterolemia between men and women, drugs for the treatment of these diseases were used more frequently in women than in men (P<0.001, each case). In contrast, use of antidiabetic drugs was similar in men and women.

There were no apparent sex-related differences in the rate of use of warfarin or aspirin at any CHADS2 score (**Table 5**).

Table 4. Medications at Baseline

	Men	Women	P value
Warfarin	73.1	72.7	0.807
Antiplatelet agents	37.9	36.0	0.328
Aspirin	32.1	30.8	0.504
Ticlopidine	5.0	5.0	0.316
Cilostazol	2.0	1.3	0.191
Antihypertensives	71.8	78.8	<0.001
ACEI	17.4	14.8	0.087
ARB	28.4	32.2	0.039
β-blockers	21.4	21.3	0.927
Calcium antagonists	36.4	42.5	0.002
Diuretics	18.6	33.4	<0.001
Lipid-lowering drugs	16.7	26.4	<0.001
Statins	14.9	23.7	<0.001
Antidiabetic drugs	10.6	10.9	0.825
Oral	8.7	8.6	0.921
Insulin	1.4	2.2	0.111

Data are % of patients.
Only major drugs for treatment of comorbidities and prevention of thromboembolism are listed (see Uchiyama et al[18] for more detailed information on medications in J-TRACE).
ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers.

Table 5. Use of Warfarin and Aspirin at Each CHADS2 Score

CHADS2 score	Warfarin use (%)		Aspirin use (%)	
	Men	Women	Men	Women
0	57.9	61.4	32.3	28.1
1	68.9	66.2	33.9	31.4
2	74.8	73.8	30.6	32.0
3	84.9	77.0	26.9	30.3
4	81.1	87.2	34.4	29.1
5	77.3	79.3	31.3	27.6
6	83.3	66.7	83.3	66.7
P value	<0.001	0.001	0.008	0.852

Warfarin usage differed significantly among CHADS2 scores in both men (P<0.001) and women (P=0.001). It increased gradually from approximately 60% to 80% as the score increased from 0 to 3 for both men and women; thereafter it reached a plateau, except in the case of women with a score of 6. Aspirin usage also differed significantly among CHADS2 scores in men (P=0.008), but not in women (P=0.852). It did not show any apparent score-dependent increase as observed in the case of warfarin usage.

Discussion

The major findings of the present study are as follows. First, there were sex-related differences in the risk factor profile and medications of patients with AF recruited in J-TRACE. Women were older and more frequently had hypertension, valvular diseases, congestive heart failure, and hypercholesterolemia than men. The prevalence of diabetes mellitus, ischemic stroke, and MI did not differ between men and women. Second, CHADS2 score was consequently slightly but significantly higher in women than in men with AF. This sex-related difference could be largely related to the higher proportion of women aged 75 years or older. Third, no sex-related differences in the use of warfarin or aspirin were observed at any CHADS2 score.

Risk Factor Profile of Patients With AF

Reports from Western countries[6–10] suggest that sex-related differences could exist in the risk factors for cardiovascular diseases of patients with AF. In the present study, mean age was higher and the prevalence of hypertension also tended to be higher in women than in men, consistent with the previous reports;[6–9] however, the prevalence of congestive heart failure was also higher in women than in men in the present study, a finding that is inconsistent with those reports from Western countries.[6–9] Notably, the prevalence of diabetes mellitus and of a prior history of ischemic stroke were not consistent.[6–9]

Cohort studies of the general population in Japan have indicated that the prevalences of hypertension and diabetes mellitus are higher in men than in women.[19–21] The prevalence of cardiac diseases was not higher in women than in men with AF,[4,5] so the higher prevalences of hypertension and congestive heart failure in women with AF found in the present study do not simply reflect the prevalence of these diseases in the general population of Japan. Valvular disease is a well-known risk factor for AF,[22] especially for Japanese women.[23] Drinking and smoking could promote the development of AF,[22–25] and were present more frequently in men than in women in the present study, as in the general population of Japan.[4,5,19–21] The electrophysiological properties of the atria differ between men and women,[26] so greater comorbidity and age might be required for AF to develop in women than in men.

Thromboembolic Risk

A sex difference in CHADS2 score was found in the present study, a finding consistent with the ATRIA study.[7] In the Euro Heart Survey the score might have been higher in women than in men, because mean age and the prevalences of hypertension, diabetes mellitus, and prior ischemic stroke were significantly higher in women than in men.[9] In some studies the levels of biomarkers of a prothrombotic state were higher in women with AF than in men with AF.[27,28] These findings could explain the inclusion of female sex as a risk factor in some schemes for predicting thromboembolic events in patients with AF.[13,14] In fact, among patients with acute stroke, embolic infarction is observed more frequently in women than in men.[29] It is difficult to determine the reasons for the sex-related difference in thromboembolic risk; however, some components of the CHADS2 score were observed more frequently in women in the ATRIA study,[7] Euro Heart Survey,[9] and in the present study.

Medications

Registry studies in Western countries have indicated that warfarin usage does not differ between men and women.[6,9] In the present study, the rate of warfarin usage did not differ between men and women as a whole nor did it differ between them at any CAHDS2 score (**Table 5**). Warfarin usage is at present not necessarily less frequent in women than in men, as reported in earlier registry[6] and community-based cohort[30] studies.

Use of aspirin and antidiabetic drugs was similar in men and women; however, drugs for hypertension and hypercholesterolemia were used more frequently by women than by men. The latter finding might reflect the sex-related differ-

ences in the prevalence of these diseases in the present study.

Study Limitations

First, enrollment of consecutive patients with stroke, MI, and AF was recommended, but may not necessarily have occurred at each participating site and this possible selection bias could have affected the present results. Second, data for subjects with AF were collected from 3 categories of J-TRACE,[17,18] possibly resulting in increased prevalences of ischemic stroke and MI. However, this might not necessarily have affected sex-related differences in the frequency of these diseases in the present study. Actually, when only patients of AF category were analyzed, the results did not differ in terms of sex-related differences in mean age, CHADS2 score, and prevalences of heart failure, hypertension, smoking, drinking habit and warfarin usage (data not shown). Third, the study design of the J-TRACE did not define the diagnostic criteria of comorbidities, including hypertension, hypercholesterolemia and others; however, data of comorbidities were collected from the medical record. If strict diagnostic criteria of comorbidities were used, the present results would not have changed greatly. Finally, the intensity of anticoagulation was not determined systematically, and follow-up data are not yet available.

Clinical Implications

Our findings indicate sex-related differences in the clinical risk factor profile of patients with AF, with the CHADS2 score slightly but significantly higher in women with AF than in men with AF in the clinical setting in Japan. Further follow-up studies are required to elucidate the effects of these sex-related differences on subsequent thromboembolic events.

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Disclosure

There is no conflict of interest to declare.

References

1. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG. Prevalence, age distribution, and gender of patients with atrial fibrillation. *Arch Intern Med* 1995; **155:** 469–473.
2. Go AS, Hylek EM, Phillips KA, Chang YC, Henault LE, Selby JV, et al. Prevalence of diagnosed atrial fibrillation in adults: National implications for rhythm management and stroke prevention: The AnTicoagulation and Risk factors In Atrial fibrillation (ATRIA) study. *JAMA* 2001; **285:** 2370–2375.
3. Majeed A, Moser K, Carroll K. Trends in the prevalence and management of atrial fibrillation in general practice in England and Wales, 1994–1998: Analysis of data from the general practice research database. *Heart* 2001; **86:** 284–288.
4. Inoue H, Fujiki A, Origasa H, Ogawa S, Okumura K, Kubota I, et al. Prevalence of atrial fibrillation in the general population of Japan: An analysis based on periodic health examination. *Int J Cardiol* 2009; **137:** 102–107.
5. Iguchi Y, Kimura K, Aoki J, Kobayashi K, Terasawa Y, Sakai K, et al. Prevalence of atrial fibrillation in community-dwelling Japanese aged 40 years or older in Japan: Analysis of 41,436 non-employee residents in Kurashiki-City. *Circ J* 2008; **72:** 909–913.
6. Humphries KH, Kerr CR, Connolly SJ, Klein G, Boone JA, Green M, et al. New-onset atrial fibrillation: Sex differences in presentation, treatment, and outcome. *Circulation* 2001; **103:** 2365–2370.
7. Fang MC, Singer DE, Chang Y, Hylek EM, Henault LE, Jensvold NG, et al. Gender differences in the risk of ischemic stroke and peripheral embolism in atrial fibrillation: The AnTicoagulation and Risk factors In Atrial fibrillation (ATRIA) study. *Circulation* 2005; **112:** 1687–1691.
8. Gomberg-Maitland M, Wenger NK, Feyzi J, Lengyel M, Volgman AS, Petersen P, et al. Anticoagulation in women with non-valvular atrial fibrillation in the stroke prevention using an oral thrombin inhibitor (SPORTIF) trials. *Eur Heart J* 2006; **27:** 1947–1953.
9. Dagres N, Nieuwlaat R, Vardas PE, Andersen D, Levy S, Cobbe S, et al. Gender-related differences in presentation, treatment, and outcome of patients with atrial fibrillation in Europe: A report from the Euro Heart Survey on atrial fibrillation. *J Am Coll Cardiol* 2007; **49:** 572–577.
10. Kerr CR, Humphries K. Gender-related differences in atrial fibrillation. *J Am Coll Cardiol* 2005; **46:** 1307–1308.
11. Friberg J, Scharling H, Gadsboll N, Truelsen T, Jensen GB. Comparison of the impact of atrial fibrillation on the risk of stroke and cardiovascular death in women versus men (the Copenhagen City Heart Study). *Am J Cardiol* 2004; **94:** 889–894.
12. Stewart S, Hart CL, Hole DJ, McMurray JJV. A population-based study of long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study. *Am J Med* 2002; **113:** 359–364.
13. Hart R, Pearce L, McBride R, Rothbart R, Asinger R. Factors associated with ischemic stroke during aspirin therapy in atrial fibrillation: Analysis of 2012 participants in the SPAF I-III clinical trials. *Stroke* 1999; **30:** 1223–1229.
14. Wang TJ, Massaro JM, Levy D, Vasa RS, Wolf PA, D'Agostino RB, et al. A risk score for predicting stroke or death in individuals with new-onset atrial fibrillation in the community: The Framingham Heart Study. *JAMA* 2003; **290:** 1049–1056.
15. Gage BF, Waterman AD, Shannon W, Boechler M, Rich MW, Radford MJ. Validation of clinical classification schemes for predicting stroke: Results from the National Register of Atrial Fibrillation. *JAMA* 2001; **285:** 2864–2870.
16. Atrial Fibrillation Investigators. Risk factors for stroke and efficacy of antithrombotic therapy in atrial fibrillation: Analysis of pooled data from five randomized controlled trials. *Arch Intern Med* 1994; **154:** 1449–1457.
17. Origasa H, Goto S, Uchiyama S, Shimada K, Ikeda Y, on behalf of the J-TRACE Investigators. The Japan Thrombosis Registry for Atrial Fibrillation, Coronary or Cerebrovascular Events (J-TRACE): A nation-wide, prospective large cohort study; the study design. *Circ J* 2008; **72:** 991–997.
18. Uchiyama S, Shibata Y, Hirabayashi T, Mihara B, Hamashige N, Kitagawa K, et al for the J-TRACE Investigators. Risk factor profiles in patients with stroke, myocardial infarction, and atrial fibrillation: The Japan Thrombosis Registry for Atrial Fibrillation, Coronary or Cerebrovascular Events (J-TRACE). *J Stroke Cerebrovasc Dis* (in press).
19. Ohsawa M, Okayama A, Okamura T, Itai K, Nakamura M, Tanno K, et al for the NIPPON DATA80 Research Group. Mortality risk attributable to atrial fibrillation in middle-aged and elderly people in the Japanese general population: Nineteen-year follow-up in NIPPON DATA80. *Circ J* 2007; **71:** 814–819.
20. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Arima H, Tanaka K, et al. Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community: The Hisayama Study. *Stroke* 2003; **34:** 2349–2354.
21. Iso H, Sato S, Kitamura A, Imano H, Kiyama M, Yamagishi K, et al. Metabolic syndrome and the risk of ischemic heart disease and stroke among Japanese men and women. *Stroke* 2007; **38:** 1744–1751.
22. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort: The Framingham Heart Study. *JAMA* 1994; **271:** 840–844.
23. Fujishima M. Cardiac diseases as risk for cerebrovascular diseases. *Junkanki Senmon-i* 1998; **6:** 19–26 (in Japanese).
24. Djousse L, Levy D, Benjamin EJ, Blease S, Russ A, Larson MG, et al. Long-term alcohol consumption and the risk of atrial fibrillation in the Framingham study. *Am J Cardiol* 2004; **93:** 710–713.
25. Heerings J, Kors JA, Hofman A, van Rooij FJA, Witteman JCM. Cigarette smoking and risk of atrial fibrillation: The Rotterdam Study. *Am Heart J* 2008; **156:** 1163–1169.
26. Tse HF, Oral H, Pelosi F, Knight BP, Strickberger SA, Morady F. Effect of gender on atrial electrophysiologic changes induced by rapid atrial pacing and elevation of atrial pressure. *J Cardiovasc Electrophysiol* 2001; **12:** 986–989.
27. Conway DS, Heeringa J, Van der Kuip DA, Chin BS, Hofman A, Witteman JC, et al. Atrial fibrillation and the prothrombotic state in the elderly: The Rotterdam study. *Stroke* 2003; **34:** 413–417.
28. Feinberg WM, Pearce LA, Hart RG, Cushman M, Cornell ES, Lip GY, et al. Markers of thrombin and platelet activity in patients with

atrial fibrillation: Correlation with stroke among 1531 participants in the Stroke Prevention in Atrial Fibrillation III study. *Stroke* 1999; **30:** 2547–2553.
29. Appelros P, Stegmayr B, Terent A. Sex differences in stroke epidemiology: A systematic review. *Stroke* 2009; **40:** 1082–1090.
30. Sudlow M, Thomson R, Thwaites B, Rodgers H, Kenny RA. Prevalence of atrial fibrillation and eligibility for anticoagulants in the community. *Lancet* 1998; **352:** 1167–1171.

Appendix 1

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ORIGINAL RESEARCH

Positional Relationship between Recurrent Intracerebral Hemorrhage/Lacunar Infarction and Previously Detected Microbleeds

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BACKGROUND AND PURPOSE: Although MBs, ICH, and LI are secondary to cerebral microangiopathy, it remains unclear whether the location of subsequent ICH/LI corresponds to the previous location of MBs. We performed this study to clarify the positional relationship between recurrent ICH/LI and previously detected MBs.

MATERIALS AND METHODS: We evaluated patients with recurrent ICH/LI who had MBs, as shown on prior T2*-weighted MR imaging. We assessed retrospectively whether the location of recurrent ICH/LI corresponded to that of the prior MB. Patients with ICH were divided into the deep ICH group and the lobar ICH group, and the positional relationship between hematoma and previously detected MBs was evaluated.

RESULTS: A total of 55 patients, including 34 with recurrent ICH and 21 with recurrent LI were evaluated. Although the location of the LI corresponded to prior MBs in only 1 patient (4.8%), the location of ICH corresponded to prior locations of MBs in 21 patients (61.8%) (OR, 32.3; 95% CI, 3.86–270.3; $P < .001$). Among the patients with ICH, the correspondence ratio was higher in the deep ICH group (19 of 24 patients, 79.2%) than in the lobar ICH group (2 of 10 patients, 20%) (OR, 15.2; 95% CI, 2.42–95.3; $P < .002$).

CONCLUSIONS: The close positional association between recurrent ICH and prior MBs suggests that MBs represent hemorrhage-prone microangiopathy. In addition, different correspondence ratios between the deep ICH group and the lobar ICH group may be attributable to their different pathogenesis.

ABBREVIATIONS: ATBI = atherothrombotic brain infarction; CAA = cerebral amyloid angiopathy; CE = cardioembolic infarction; CI = confidence interval; DWI = diffusion-weighted imaging; ICH = intracerebral hemorrhage; LI = lacunar infarction; MB = microbleed; OR = odds ratio

MBs present as homogeneous round lesions with signal-intensity loss on gradient-echo T2*-weighted MR images. Pathologically, they represent hemosiderin deposits,[1,2] associated with small-vessel disease.

Previous studies have shown that MBs are observed more frequently in patients with ICH compared with patients with ischemic stroke.[3,4] Among patients with ischemic stroke, they are observed more frequently in patients with LI, which is based on small-vessel disease, compared with patients with ATBI or CE.[5,6] In addition, MBs are more prevalent among patients with recurrent stroke compared with patients with their first stroke.[4] Previous studies have also shown that the presence of MBs is an important risk factor for the occurrence of subsequent stroke, particularly hemorrhagic stroke.[7-9]

The topologic association, however, between the location of MBs and that of subsequent stroke is poorly understood. Although previous reports described the association between the hematoma and the distribution of MBs at the onset of ICH,[10,11] a few case reports[12,13] and several cases described in a prospective study that was performed for other purposes[7,14] have reported that the subsequent ICH occurred in the same lesion in which prior MBs were detected. Moreover, to our knowledge, topologic association in patients with LI has not been reported.

This retrospective study was designed to clarify the positional association between recurrent ICH/LI and previously detected MBs in a relatively large number of patients.

Materials and Methods

Study Design and Patients

We evaluated consecutive patients with acute recurrent ICH/LI who were admitted to our hospital from June 2003 to June 2008. Among them, the patients who had asymptomatic MBs identified on 1.5T gradient-echo T2*-weighted MR imaging, which was performed at the time of the prior stroke event, were included in the study. Patients with CE, ATBI, or undetermined classification were excluded. The diagnosis of acute stroke was made on the basis of neurologic and neuroradiologic examinations. Recurrent stroke was classified into ischemic stroke and ICH, and ischemic stroke was further subclassified as ATBI, CE, and LI, according to the diagnostic criteria based on the National Institute of Neurologic Disorders and Stroke Ad Hoc Committee Classification of Cerebrovascular Disease III.[15] Of the 55 patients included, 34 had recurrent ICH and 21 had recurrent LI.

The location of recurrent ICH was assessed by using CT, and the location of recurrent LI was assessed by DWI and apparent diffusion

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Table 1: Characteristics of patients with ICH and LI

Characteristic	ICH (n = 34)	LI (n = 21)	P Value[a]
Demographic data			
Median age, (yr) (range)	69.5 (51–84)	72 (57–89)	.020
Male sex, No. (%)	25 (73.5)	13 (61.9)	.365
Vascular risk factors			
Hypertension (%)	97.0	100	1.000
Diabetes mellitus (%)	24.2	31.6	.746
Hyperlipidemia (%)	45.2	52.6	.608
Antithrombotic therapy (%)	56.3	78.9	.135
Prior stroke subtype, No. (%)			
ICH	13 (38.2)	2 (9.5)	.029
LI	12 (35.3)	18 (85.7)	.005
ATBI	4 (11.8)	1 (5.9)	.639
CE	5 (14.7)	0 (0)	.144
No. of MBs, median (range)	12.5 (1–73)	6 (1–83)	.070
Time from prior stroke, median day (range)	247.5 (14–1873)	179 (3–860)	.188
Correspondence to MBs, No. (%)	21 (61.8)	1 (4.8)	<.001

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used.

coefficient maps. We assessed, retrospectively, whether the location of recurrent ICH/LI corresponded to that of the previously detected MBs. Furthermore, patients with ICH were divided into the deep ICH group (hematoma present in the thalamus, the putamen, the pons, and the cerebellum) and the lobar ICH group (hematoma in a subcortical location), and the positional relationship between the hematoma and previously detected MBs was evaluated. There were no patients with a recurrent caudate hemorrhage in the present study. Previous antithrombotic therapy, the number of previously detected MBs, and the duration from the prior stroke to the recurrence were also evaluated in each patient. In patients with recurrent ICH, the hemorrhage volume was also evaluated. The study protocol for the chart review was approved by our institutional review board.

Vascular Risk Factors

We assessed vascular risk factors such as history of previous stroke and the presence of hypertension, diabetes mellitus, or hyperlipidemia. "Hypertension" was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg, which were measured with an automated cuff-oscillometric device at least 2 times in the outpatient department before recurrence of stroke, or current medical treatment for hypertension. "Diabetes mellitus" was defined as a glycosylated hemoglobin A_{1c} concentration of ≥6.5% or current use of hypoglycemic agents. "Hyperlipidemia" was defined as a low-attenuation lipoprotein cholesterol level of ≥140 mg/dL or current cholesterol-lowering therapy. We also recorded the prevalence of antithrombotic therapy before occurrence of the recurrent stroke in each patient.

Neuroradiologic Examinations

All patients were examined by using a 1.5T clinical MR imaging unit (Magnetom Symphony; Siemens, Erlangen, Germany) with a section thickness of 5 mm and a 1.5-mm gap between sections. We used axial T2*-weighted gradient-echo sequences (TR/TE, 800/26 ms; flip angle, 20°; FOV, 230 × 230; matrix, 192 × 256) to detect MBs at the onset of the prior stroke. In addition, at the onset of the recurrent stroke, we also performed axial DWI with single-shot echo-planar spin-echo sequences (TR/TE, 5300/135 ms; FOV, 196 × 261; matrix, 80 × 128; b-values, 0 and 1000/mm^2) to evaluate the location of recurrent LI, and we performed axial head CT to evaluate the location and the volume of recurrent ICH. MBs were defined as homogeneous round lesions with a diameter of ≤5 mm characterized by signal-intensity loss on T2*-weighted MR images. Signal-intensity-loss lesions in the globus pallidum (which likely represented calcification) and the subarachnoid space (which likely represented adjacent pial vessels) were excluded. Intracerebral lesions were also excluded if they had a hemorrhagic component associated with tumor, arteriovenous malformation, cavernous hemangioma, or trauma.

"Corresponding" or "correspondence" was used if the location of MBs detected on prior T2*-weighted MR imaging was involved in the ICH detected on CT or the LI detected on DWI at the onset of recurrent stroke. Two of the authors (Y.S., H.N.) without detailed knowledge of the patients' clinical profiles retrospectively compared the same section of each film and determined the correspondence of MBs with subsequent stroke. In addition, we calculated the hemorrhage volume with the ABC/2 method, in which A is the greatest diameter on the largest hemorrhage section, B is the diameter perpendicular to A, and C is the approximate number of axial sections with hemorrhage multiplied by the section thickness.[16]

Statistical Analysis

For the cases of recurrent ICH versus LI and deep brain versus lobar ICH, the χ^2 test or Fisher exact test for independence was used for comparison of sex ratio, hypertension, diabetes mellitus, hyperlipidemia, antithrombotic therapy, and correspondence between prior MBs and recurrent stroke for each group. The Student t test was used for comparison of age at the time of recurrent stroke. The Mann-Whitney U test was used for comparison of the hemorrhage volume, the number of previously detected MBs, and the time from prior stroke to the recurrence in each ICH group. $P < .05$ was considered significant. The Statistical Package for the Social Sciences, Version 16.0 for Windows (SPSS, Chicago, Illinois) was used for statistical analysis.

Results

Baseline Data

Of the 55 patients included in this study, 34 had recurrent ICH (25 men and 9 women) and 21 patients had recurrent LI (13 men and 8 women). The patients with ICH (median age, 69.5 years; range, 51–84 years) were younger compared with the patients with LI (median age, 72 years; range, 57–89 years;

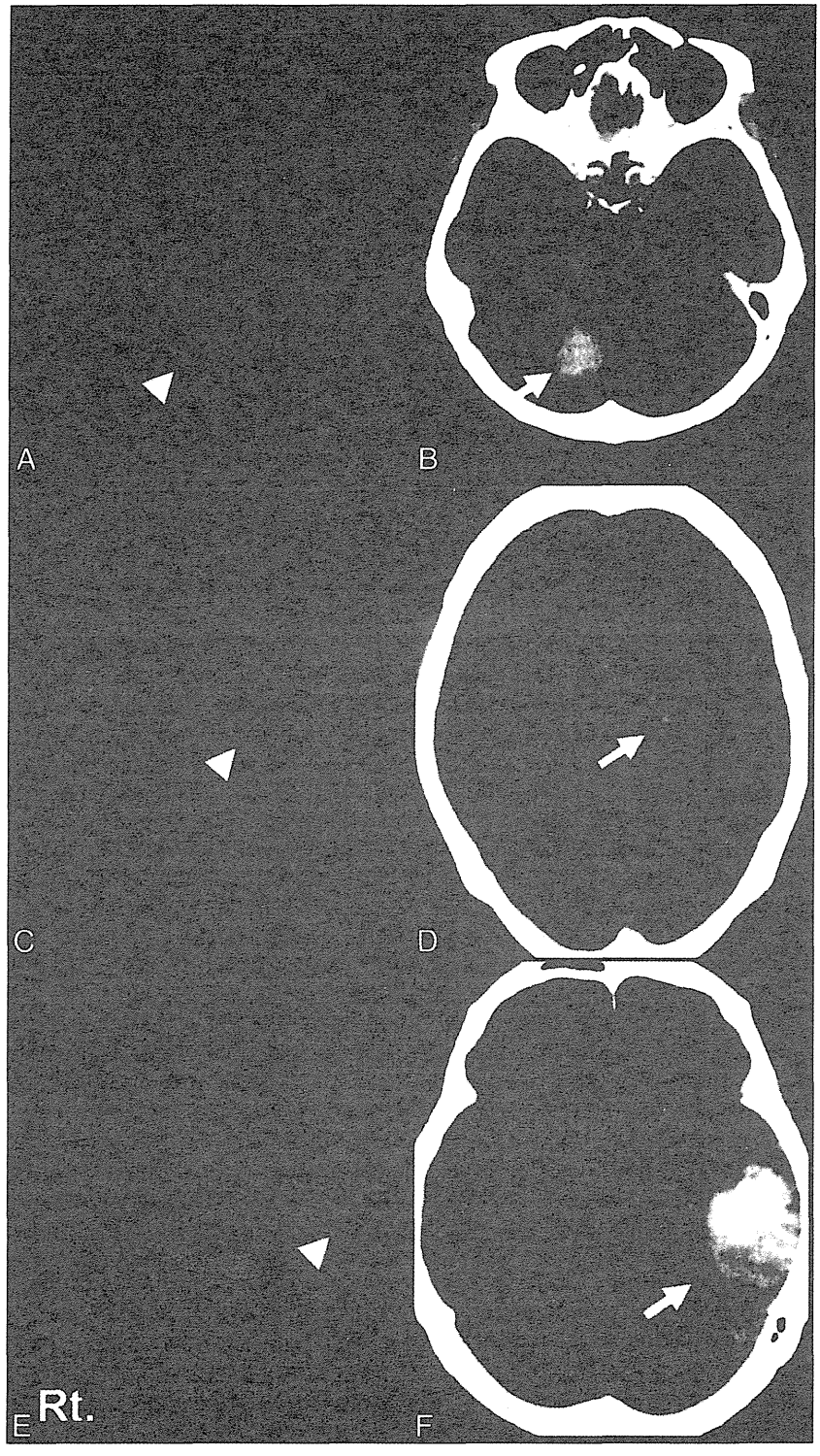

Fig 1. Representative cases. T2*-weighted MR image (*A*) and CT scan (*B*) in an 84-year-old patient. Recurrent right cerebellar hemorrhage (*arrow*) corresponds to the location of MBs detected 9 months before (*arrowhead*). T2*-weighted MR image (*C*) and CT scan (*D*) in an 80-year-old patient. Recurrent left thalamic hemorrhage (*arrow*) corresponds to the location of MBs detected 35 months before (*arrowhead*). T2*-weighted MR image (*E*) and CT scan (*F*) in an 85-year-old patient. Recurrent left lobar hemorrhage (*arrow*) corresponds to the location of MBs detected 3 months before (*arrowhead*).

$P = .020$). Other demographic and clinical data are shown in Table 1.

Positional Relationship between Recurrent ICH/LI and Previously Detected MBs. We evaluated the positional relationship between recurrent ICH/LI and previously detected MBs. In the recurrent ICH group, hematoma corresponded to the prior MBs in 21 of 34 patients (61.8%). Representative cases are shown in Fig 1. In contrast, LI corresponded to the

Table 2: Characteristics of corresponding and noncorresponding groups in patients with ICH

Characteristic	Corresponding (n = 21)	Noncorresponding (n = 13)	P Value[a]
Demographic data			
Median age, (yr) (range)	70 (51–84)	62 (55–78)	.188
Male sex, No. (%)	17 (81.0)	8 (61.5)	.151
Vascular risk factors			
Hypertension (%)	100	92.3	.934
Diabetes mellitus (%)	25.0	30.8	.681
Hypercholesterolemia (%)	26.3	61.5	.071
Antithrombotic therapy (%)	42.1	76.9	.075
Prior stroke subtype, No. (%)			
ICH	8 (38.1)	5 (35.7)	.886
LI	9 (42.9)	3 (21.4)	.282
ATBI	1 (4.8)	3 (21.4)	.279
CE	3 (14.3)	2 (14.3)	1.000
Hemorrhage volume, median (range) (cm^3)	15.1 (0.36–162)	3.43 (0.16–58.4)	.077
No. of MBs, median (range)	16 (4–73)	4 (1–49)	.014
Time from prior stroke, median day (range)	263 (58–1873)	150 (14–1407)	.748

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used.

prior MBs in only 1 of 21 patients (4.8%) in the recurrent LI group. The correspondence ratio was, therefore, higher in the recurrent ICH group than in the recurrent LI group (OR, 32.3; 95% CI, 3.86–270.3; $P < .001$). The number of MBs and the time from prior stroke to the recurrent stroke were equivalent between the recurrent ICH group and the recurrent LI group (Table 1).

Among the ICH group, the number of MBs was higher in the "corresponding" group (median, 16; range, 4–73) than in the "noncorresponding" group (median, 4; range, 1–49; P = .001). The hemorrhage volume and the time from prior stroke were equivalent between both groups. Vascular risk factors, antithrombotic therapy, and prior stroke subtype were also equivalent between both groups (Table 2).

We also evaluated the association between the initial stroke subtype and correspondence between MB and stroke in the patients with recurrent ICH. Of the 34 patients in the recurrent ICH group, 13 patients had prior ICH and 21 had prior ischemic stroke. Among them, hematoma corresponded to the prior MBs in 8 of 13 patients with prior ICH (61.5%) and 13 of 21 patients with prior ischemic stroke (61.9%). The corresponding ratio was equivalent between the patients with prior ICH and the patients with prior ischemic stroke (P = .98).

Positional Relationship between Recurrent ICH and Previously Detected MBs in the Deep ICH Group versus the Lobar ICH Group. We evaluated the positional relationship between recurrent ICH and previously detected MBs for each type of hematoma (deep ICH versus lobar ICH). In the deep ICH group, hematoma corresponded to the prior MBs in 19 of 24 cases (79.2%) including 10 of 11 cases (90.0%) of thalamic hemorrhage, 6 of 7 cases (85.7%) of putaminal hemorrhage, 2 of 4 cases (50.0%) of cerebellar hemorrhage, and 1 of 2 cases (50.0%) of pontine hemorrhage (Fig 2). In contrast, in the lobar ICH group, hematoma corresponded to the prior MBs in only 2 of 10 patients (20.0%) (Fig 2). Among the patients with ICH, the correspondence ratio was higher in the deep ICH group than in the lobar ICH group (OR, 15.2; 95% CI, 2.42–95.3; $P < .002$). The hemorrhage volume, number of MBs, and the time from prior stroke to the recurrent ICH were equivalent between both groups (Table 3).

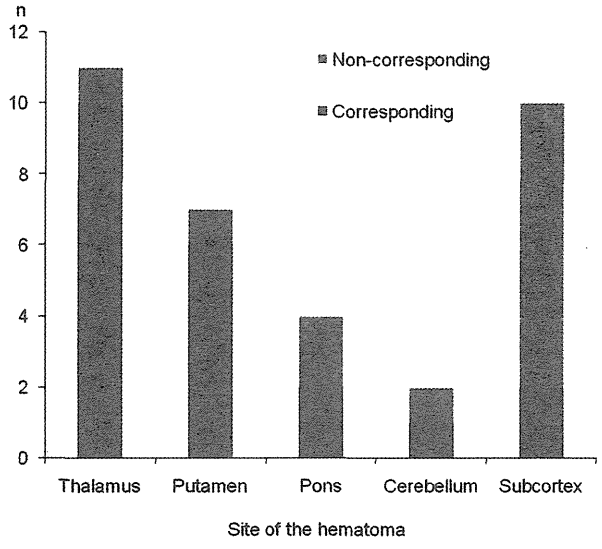

Fig 2. Correspondence of MBs in each part of the hematoma. The correspondence ratio was higher in the deep ICH group, particularly in thalamic and putaminal hemorrhage, than in the lobar ICH group.

Among the deep ICH group, the number of MBs in the whole brain and in the gray matter (thalamus, putamen, and caudate nucleus) was higher in the corresponding group (median, 16; range, 4–56; and median, 8; range, 3–28) than in the noncorresponding group (median, 4; range, 1–11; and median, 2; range, 1–8; P = .003 and P = .015). The time from prior stroke was equivalent between both groups. Vascular risk factors and prior stroke subtype were also equivalent between both groups. The rate of antithrombotic therapy was significantly higher in the noncorresponding group than in the corresponding group (Table 4).

Discussion

We found that the correspondence ratio was higher in patients with recurrent ICH than in patients with recurrent LI. In addition, among the patients with recurrent ICH, the correspondence ratio was higher in the deep ICH group, particularly in hemorrhage involving the putamen and thalamus, compared with the lobar ICH group.

Table 3: Characteristics of deep ICH and lobar ICH groups

Characteristic	Deep ICH (n = 24)	Lobar ICH (n = 10)	P Value[a]
Demographic data			
Median age (yr) (range)	69.5 (51–84)	66 (55–84)	.694
Male sex, No. (%)	17 (70.8)	8 (80.0)	.692
Vascular risk factors			
Hypertension (%)	100	90.0	.303
Diabetes mellitus (%)	26.1	20.0	1.000
Hyperlipidemia (%)	45.5	44.4	1.000
Antithrombotic therapy (%)	50.0	77.8	.237
Prior stroke subtype, No. (%)			
ICH	9 (37.5)	4 (40.0)	1.000
LI	11 (45.8)	1 (10.0)	.061
ATBI	1 (4.2)	3 (30.0)	.067
CE	3 (12.5)	2 (20.0)	.618
Hemorrhage volume, median (range) (cm^3)	6.92 (0.16–66.9)	30.3 (0.69–162)	.287
No. of MBs, median (range)	13 (1–56)	8 (1–73)	.304
Time from prior stroke, median day (range)	292.5 (14–1873)	187.5 (79–1033)	.696
Correspondence to MBs, No. (%)	19 (79.2)	2 (20.0)	.002

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used.

Table 4: Characteristics of the corresponding and noncorresponding groups in the deep ICH group

Characteristic	Corresponding (n = 19)	Noncorresponding (n = 5)	P Value[a]
Demographic data			
Median age, (yr) (range)	70 (51–84)	67 (60–78)	.996
Male sex, No. (%)	15 (78.9)	2 (40.0)	.126
Vascular risk factors			
Hypertension (%)	100	100	-
Diabetes mellitus (%)	16.7	60.0	.078
Hypercholesterolemia (%)	29.4	60.0	.309
Antithrombotic therapy (%)	35.3	100	.035
Prior stroke subtype, No. (%)			
ICH	7 (36.8)	2 (40.0)	1.000
LI	9 (47.4)	2 (40.0)	1.000
ATBI	0 (0)	1 (20.0)	.208
CE	3 (15.8)	0 (0)	1.000
No. of MBs, median (range)			
In the whole brain	16 (4–56)	4 (1–11)	<.001
In the deep gray matter	8 (3–28)	2 (1–8)	.015
Time from prior stroke, median day (range)	322 (58–1873)	99 (14–1407)	.746

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used.

Only a few case reports[12,13] and several cases described in prospective studies performed other purposes[7,14] found that the subsequent ICH occurred in the same lesion in which prior MBs were detected. The present study is the first report focusing on the positional relationship between the subsequent ICH and the prior detected MBs in a relatively large number of patients.

Pathologically, MBs represent hemosiderin deposits that result from the fragility of small vessels in conditions such as lipohyalinosis, CAA, or arteriosclerosis.[1,2] The presence of MBs is closely associated with small-vessel diseases such as ICH and LI,[5,6] and it has been reported to be an important risk factor for subsequent stroke, particularly hemorrhagic stroke.[7-9]

The difference in correspondence ratios between ICH and LI may result from the difference in topology among ICH, LI, and MBs. Previous studies showed that MBs tend to be frequently present at the site of hypertensive ICH.[17,18] In contrast, MBs are seldom detected in the posterior limb of the internal capsule or the corona radiata,[18] which are the frequent sites of LI. This topographic difference may explain the discrepancy of the correspondence ratios between ICH and LI. However, it remains unclear why MBs are seldom detected in the frequent sites of LI and, furthermore, why the locations of prior MBs and recurrent LI do not coincide in other brain regions, even though both MBs and LI are based on microangiopathy. The close topologic association between prior MBs and recurrent ICH but not recurrent LI indicates that MBs are a form of small-vessel disease that is bleeding-prone.

The present study also reveals that the correspondence ratio in the deep ICH group was higher than that in the lobar ICH group, though the hemorrhage volume and the number of MBs were equivalent between both groups. Our findings may support the results of the Rotterdam Scan Study that MBs in a deep or infratentorial location were associated with hypertensive or atherosclerotic microangiopathy, whereas lobar MBs were related to CAA.[19] In the deep ICH group, close topologic association of prior MBs with subsequent ICH, particularly in the putamen and thalamus, suggests that subsequent hemorrhage may result from rerupture of microangiopathic vessels, such as those with lipohyalinosis in the deep brain area, which had been detected as MBs. In addition, the

higher number of MBs in the deep gray matter in the corresponding group suggests that MBs in this area may be a marker of the ongoing hypertensive microangiopathy and at risk for further subsequent ICH.

In contrast, the present study reveals the lower corresponding ratio between the prior MBs and subsequent ICH in the lobar ICH group. A recent pathologic study in patients with CAA suggested that the patients with many MBs demonstrated thicker amyloid-positive vessels than those with few MBs; therefore, CAA-related hemorrhage and MBs are based on different pathologies.[20] The CAA-related hemorrhage may result from rupture of amyloid-positive vessels, which are different from the vessels detected as MBs; the pathologic difference between ICH and MBs in CAA may result in the lower corresponding ratio in the lobar ICH group in the present study. However, we could not determine exactly whether the lobar hemorrhage resulted from CAA or hypertension because no patients enrolled in the present study were examined pathologically. This point is 1 of the limitations of the present study.

The other limitations should be noted. ICHs are often sizeable (particularly compared with LIs) and might, therefore, appear to coincide with a prior MBs simply because they cover a large volume of brain. It is even possible that deep ICHs, by occurring in a more confined anatomic territory than lobar ICHs, might be predisposed to coincide with prior MBs in the same territory. On the other hand, there was no difference in the hemorrhage volume between the corresponding group and the noncorresponding group overall in patients with ICH and between the deep ICH group and the lobar ICH group. Therefore, the effects of the hemorrhage volume for the corresponding ratio between the location of subsequent ICH and that of previously detected MBs may be excluded in the patients with ICH. In addition, although the corresponding ratio for patients with putamen/thalamic hemorrhages appeared to be higher than that in patients with pontine/cerebellar hemorrhages, this could be an aberration due to the small number of patients with pontine/cerebellar hemorrhage in our study. To clear up these limitations and confirm our results, we should perform prospective studies with a larger group of patients.

Conclusions

The close association between recurrent ICH and the location of previously detected MBs, especially in the putamen or thalamus, suggests that MBs represent hemorrhage-prone microangiopathy. In addition, the topologic distribution of MBs may be meaningful imaging information because the risk of subsequent ICH occurs in the same lesion in which MBs were previously detected. However, it still remains unclear whether the subsequent ICH in the location of previously detected MBs could be prevented with strict hypertension treatment or careful antithrombotic therapy, and prospective studies are needed to clarify these points.

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References

1. Fazekas F, Kleinert R, Roob G, et al. **Histopathologic analysis of foci of signal loss in gradient-echo T2* -weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds.** *AJNR Am J Neuroradiol* 1999;20:637–42
2. Tanaka A, Ueno Y, Nakayama Y, et al. **Small chronic hemorrhages and ischemic lesions in association with spontaneous intracerebral hematomas.** *Stroke* 1999;30:1637–42
3. Naka H, Nomura E, Wakabayashi S, et al. **Frequency of asymptomatic microbleeds on T2* -weighted MR images of patients with recurrent stroke: association with combination of stroke subtypes and leukoaraiosis.** *AJNR Am J Neuroradiol* 2004;25:714–19
4. Cordonnier C, Salman RA, Wardlaw J. **Spontaneous brain microbleeds: systemic review, subgroup analysis and standards for study design and reporting.** *Brain* 2007;130:1988–2003
5. Kato H, Izumiyama M, Izumiyama K, et al. **Silent cerebral microbleeds on T2*-weighted MRI: correlation with stroke subtype, stroke recurrence, and leukoaraiosis.** *Stroke* 2002;33:1536–40
6. Koennecke HC. **Cerebral microbleeds on MRI: prevalence, associations, and potential clinical implications.** *Neurology* 2006;66:165–71
7. Fan YH, Zhang L, Lam WW, et al. **Cerebral microbleeds as a risk factor for subsequent acute ischemic stroke.** *Stroke* 2003;34:2459–62
8. Imaizumi T, Horita Y, Hashimoto Y, et al. **Dotlike hemosiderin spots on T2*-weighted magnetic resonance imaging as a predictor of stroke recurrence: a prospective study.** *J Neurosurg* 2004;101:915–20
9. Naka H, Nomura E, Takahashi T, et al. **Combinations of the presence or absence of cerebral microbleeds and advanced white matter hyperintensity as predictors of subsequent stroke types.** *AJNR Am J Neuroradiol* 2006;27:830–35
10. Roob G, Lechner A, Schmidt R, et al. **Frequency and location of microbleeds in patients with primary intracerebral hemorrhage.** *Stroke* 2000;31:2665–69
11. Lee SH, Bae HJ, Kwon SJ, et al. **Cerebral microbleeds are regionally associated with intracerebral hemorrhage.** *Neurology* 2004;62:72–76
12. Kidwell CS, Saver JL, Villablanca JP, et al. **Magnetic resonance imaging detection of microbleeds before thrombolysis: an emerging application.** *Stroke* 2002;33:95–98
13. Vernooij MW, Heeringa J, de Jong GJ, et al. **Cerebral microbleed preceding symptomatic intracerebral hemorrhage in a stroke-free person.** *Neurology* 2009;72:763–65
14. Huang Y, Cheng Y, Wu J, et al. **Cilostazol as an alternative to aspirin after ischaemic stroke: a randomised, double-blind, pilot study.** *Lancet Neurol* 2008;7:494–99. Epub 2008 May 2
15. **Special report from the National Institute of Neurological Disorders and Stroke: classification of cerebrovascular diseases III.** *Stroke* 1990;21:637–76
16. Kothari RU, Brott T, Broderick JP, et al. **The ABCs of measuring intracerebral hemorrhage volumes.** *Stroke* 1996;27:1304–05
17. Lee SH, Kwon SJ, Kim KS, et al. **Topographical distribution of pontocerebellar microbleeds.** *AJNR Am J Neuroradiol* 2004;25:1337–41
18. Lee SH, Kwon SJ, Kim KS, et al. **Cerebral microbleeds in patients with hypertensive stroke: topographical distribution in the supratentorial area.** *J Neurol* 2004;251:1183–89
19. Vernooij MW, van der Lugt A, Ikram MA, et al. **Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study.** *Neurology* 2008;70:1208–14
20. Greenberg SM, Nandigam RN, Delgado P, et al. **Microbleeds versus macrobleeds: evidence for distinct entities.** *Stroke* 2009;40:2382–86

Histologic characterization of mobile and nonmobile carotid plaques detected with ultrasound imaging

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Objectives: **Although mobile plaques in the carotid arteries detected by duplex ultrasound imaging are considered to cause unstable neurologic symptoms such as crescendo transient ischemic attack or progressive stroke, the histology of mobile plaques has not been sufficiently documented. This study examined the histopathologic features of mobile plaques of the carotid artery and compared the histopathology between mobile and nonmobile plaques.**

Methods: **Of 228 carotid plaques assessed by preoperative carotid ultrasound imaging, 21 (9.3%) were diagnosed as mobile symptomatic plaques. Of these, 18 were intact after excision by endarterectomy and enrolled for histologic examination. From the remaining 207 nonmobile plaque specimens, 17 nonmobile but symptomatic plaque specimens were extracted for histologic comparison. An investigator blinded to the ultrasound findings assessed both plaque specimens for fibrous cap thickness, fibrous cap rupture, fibrous cap area, necrotic core size, inflammatory cells, intraplaque hemorrhage, and mural thrombus. Clinical data, including progressive ischemic symptoms after admission, were also examined.**

Results: **Progressive ischemic symptoms were more frequently seen in patients with mobile plaques than in those with nonmobile plaques (33.3% vs 0%, $P = .02$). The ratio of the cross-sectional area of the necrotic core to that of the entire plaque was significantly larger for mobile plaques than for nonmobile plaques (mean, 0.660 vs 0.417, $P < .0001$). Mural thrombus was more prevalent among mobile plaques (89%) than among nonmobile plaques (59%), but the difference was not significant ($P = .06$). The median minimum thickness of the fibrous cap was extremely small in both groups (80 μm in mobile plaques and 100 μm in nonmobile plaques, $P = .33$).**

Conclusions: **The histologic characteristics of mobile carotid plaques are different from those of nonmobile symptomatic plaques, especially in the area of the necrotic core. This histologic difference may partly explain the unstable neurologic presentations of patients with mobile carotid plaques. (J Vasc Surg 2011;53:977-83.)**

Mobile components in symptomatic carotid plaques, as detected with a duplex ultrasound scan using the recently developed high-resolution real-time B-mode system, are assumed to cause unstable neurologic symptoms such as crescendo transient ischemic attack or progressive stroke. These types of plaque with mobility have been denoted variously in several case reports as "mobile plaques,"[1] "floating plaques,"[2-4] "mobile thrombi,"[5] or "floating thrombi."[6,7] Some authors have emphasized the high potential of the mobile plaque to cause recurrence of ischemic attacks within a short period.[5,8,9] They have also speculated that plaque disruption and mural thrombus resulted in mobile plaques.[5,6]

Previous reports have not, however, sufficiently documented the mechanism of that mobility or the histologic feature of such plaques. We hypothesized that certain histologic differences may exist between mobile and nonmobile symptomatic carotid plaques as long as clinical symptoms caused by mobile plaques are more unstable than those by nonmobile plaques. To confirm this hypothesis, we compared the prevalence of several histologic factors between mobile and nonmobile plaques in symptomatic patients, with the examination of clinical data including progressive ischemic symptoms after admission.

METHODS

This study was performed in accordance with the ethical guidelines of our institution and included patients' informed consent.

Plaque selection. Between April 2003 and March 2008, 228 carotid plaques were excised by carotid endarterectomy (CEA) at the National Cerebral and Cardiovascular Center, Osaka, Japan. All patients had been assessed with preoperative carotid ultrasound imaging, and 21 symptomatic patients (9.3%) had been diagnosed with mobile plaques. The study excluded 3 of 21 mobile plaque specimens after the histologic examination because they

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were damaged during plaque excision. From the remaining 207 nonmobile plaques, 20 symptomatic plaques were randomly extracted for histologic comparison. After the histologic examination, the study excluded 3 of the 20 nonmobile plaque specimens because they were too damaged. The remaining 35 plaque specimens, comprising 18 mobile plaques and 17 nonmobile plaques, were used in this study.

Clinical data. We reviewed clinical data of the 35 patients with excised plaques. Their symptoms at admission were classified into four categories: amaurosis fugax, transient ischemic attack (TIA), transient symptom associated with infarction (TSI), and stroke. Amaurosis fugax was defined as a transient ipsilateral blindness or visual field defect. TIA was defined as a transient neurologic symptom that lasts <24 hours without any evidence of brain infarction confirmed by diffusion-weighted images (DWI) in magnetic resonance imaging. TSI was defined as a transient neurologic symptom that lasts <24 hours with evidence of brain infarction, which is supposed to have higher in-hospital recurrent ischemic rate than TIA.[10] Stroke was confirmed by positive findings in the territory of the ipsilateral carotid artery on DWI. Progressive symptoms were also recorded when the patient experienced a recurrence and worsening of neurologic symptoms after admission, with an increase of ischemic lesions confirmed by DWI.

The degree of carotid stenosis was measured by digital subtraction angiography according to the method used in the North American Carotid Surgery Trial.[11] The other clinical data recorded were age, sex, treatment for hypertension, treatment for diabetes, treatment for hyperlipidemia, smoking within the preceding year, statin administration, and aspirin administration. Median intervals from the last ischemic event to CEA and then from the last ultrasound imaging to CEA were also examined.

Ultrasound imaging. All patients underwent preoperative carotid ultrasound scanning ≤1 month before CEAs using a commercially available, real-time 2-dimensional device equipped with a 7.5-MHz transducer. B-mode scans, B-mode scans with color Doppler imaging, and pulsed-Doppler scans were routinely performed. If a stroke physician suspected the presence of mobile plaques on duplex ultrasound imaging, the images would be recorded as video files. Two skilled stroke physicians, who had no previous knowledge of the patient's clinical information, including a coauthor (K.N.), reviewed video files and made a final diagnosis of mobile plaques. The findings of the mobile plaques were defined and classified as follows:

1. Mobile components that are localized at the surface of the plaque and that rise and fall in a manner inconsistent with or exceeding arterial pulsatile wall motion (jellyfish sign[12]),
2. Mobile components inside the plaque that change slowly and irregularly like viscous liquid (liquefaction sign),
3. Movements localized within an ulcer's inner surface (Fig 1; Video 1, online only), and

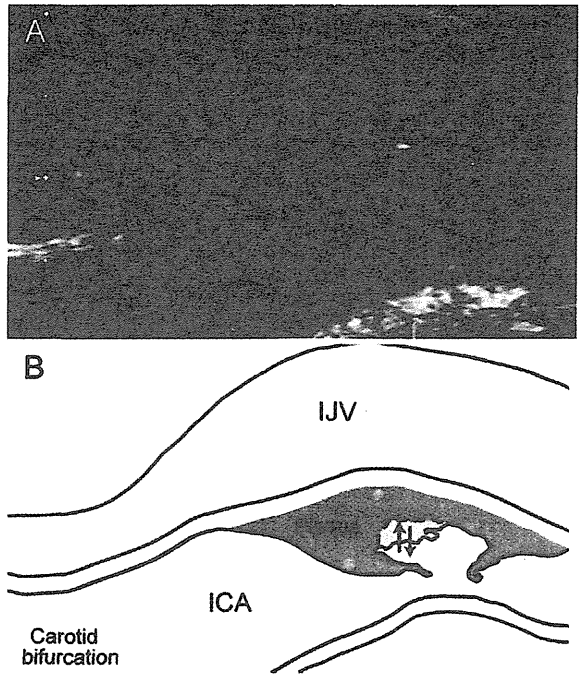

Fig 1. A, A longitudinal duplex ultrasound image of a mobile plaque demonstrates an ulcerated plaque in the extracranial internal carotid artery. **B**, A schema representing the mobile component in the plaque: an ulcer's inner surface rose and fell according to the pulsation, as indicated with *arrows*, which was defined as "movements localized within an ulcer's inner surface" (see also Video 1, online only). *ICA*, Internal carotid artery; *IJV*, internal jugular vein.

4. Movements of protuberances (Fig 2; Video 2, online only).

Plaque excision. General anesthesia was initiated, and CEA was performed using an operating microscope and somatosensory evoked potential monitoring to selectively place the shunt. For some cases with a mobile plaque, each surrounding artery (including the common, external, and internal carotid arteries) was clamped as soon as it was exposed to minimize the risk of distal embolism caused by the manipulation of the internal carotid artery. Upon cross-clamping, the common carotid artery was incised with scalpels to determine the dissection plane, usually made at the level of the internal elastic membrane, under the operating microscope. A microdissector was inserted meticulously, not to disturb the cleavage plane, until the distal end of the plaque and the patent lumen of the distal internal carotid artery were ascertained. The distal and proximal edges of the plaque were cut and finally pulled out from the orifice of the external carotid artery. In this way, most of the carotid plaque could be removed en bloc with minimum surgical trauma. If a cut penetrated the surface of the specimen to the lumen, it could be judged in the histologic examination that the cleavage resulted from surgical trauma, not plaque rupture.

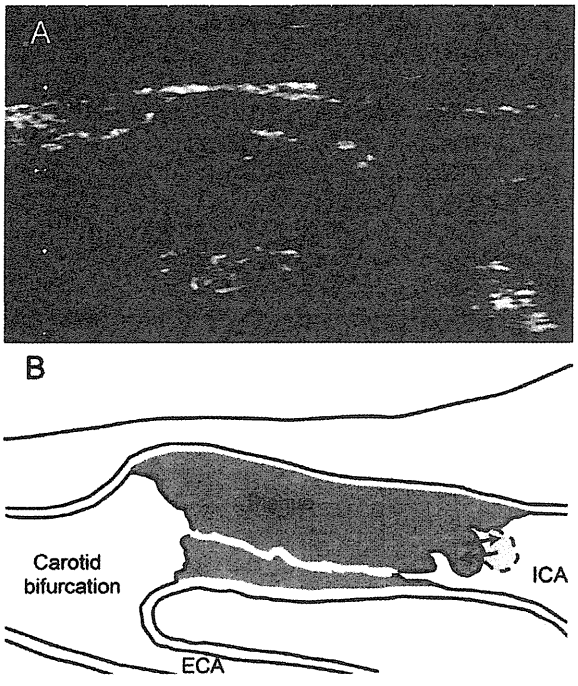

Fig 2. A, A longitudinal duplex ultrasound image demonstrates a massive mobile plaque almost occluding the internal carotid artery (*ICA*) and a protuberance from the distal end of the plaque. **B**, A schema representing the shaking movement of the protuberance (*arrows*): the original video also revealed a mobile component inside the plaque that changed slowly and irregularly like viscous liquid (liquefaction sign, see also Video 2, online only). *ECA*, External carotid artery.

Histopathology. The excised plaques were immediately fixed in Histochoice fixative (Amresco, Cleveland, Ohio) for 48 hours and decalcified by ethylenediaminetetraacetic acid (EDTA). To preserve the immunoreactivity, we used Histochoice for fixation and EDTA for decalcification of specimens before embedding in paraffin blocks.[13] Each plaque was sectioned transversely at the carotid bifurcation, and further sections were taken at 5-mm intervals along the length of internal carotid arteries for embedding in paraffin.[14] Adjacent 5-μm transverse sections were stained with hematoxylin and eosin, elastin van Gieson, Masson trichrome, and von Kossa. When a certain section seemed near the plaque rupture site, additional subserial slices were performed to avoid skipping focal instability. For immunohistochemistry analyses, we performed immunostaining for T cell (CD3, DAKO, Glostrup, Denmark), macrophages (CD68, DAKO). Immunostaining with glycophorin A (CD235a, DAKO) was also performed to detect intraplaque hemorrhage.[15] An experienced cardiovascular pathologist (H.I.) histologically examined all sections without any knowledge of clinical details and findings of carotid ultrasound imaging.

The histologic features of plaques assessed in this study were minimum cap thickness, prevalence of the rupture of fibrous cap and ulceration, necrotic core size, quantity of inflammatory cells (including macrophages and lymphocytes), degree of intraplaque hemorrhage, and prevalence of mural thrombus. Minimum cap thickness was defined as the thinnest part of the fibrous cap in total cross-sections of each plaque measured by a manometer attached to the microscope.[16] Plaque rupture, a break in the fibrous cap, was recorded when there was clear interaction between the lipid core and the lumen, usually at a point of thinning and inflammation and when the break in the cap did not seem to have been created during surgery (Fig 3, *A*). A necrotic core was defined as an amorphous material containing cholesterol crystals (Fig 3, *B*).[17]

To measure the area of necrotic core, we sampled three cross-sections: on the carotid bifurcation, 5 mm distal to the bifurcation, and 10 mm distal to the bifurcation. On each cross-section, the necrotic core and the entire plaque area were measured by WinROOF 5.0 morphometry software (Mitani Co, Kanazawa, Japan), and the ratio of the mean cross-sectional area of the necrotic core to that of the entire plaque area was calculated. We also measured the actual area of the fibrous cap for mobile and nonmobile plaques on the three cross-sections, and the median value of the fibrous cap area in each plaque was calculated.

A "recent" intraplaque hemorrhage was recorded when an area of erythrocytes within the plaque caused disruption of plaque architecture, whereas an "old" intraplaque hemorrhage was recorded when evidence showed organized hemorrhage with the accumulation of hemosiderin-laden macrophages or iron deposits on plaque connective tissue.[18] Old intraplaque hemorrhage was also recorded when the ratio of the glycophorin A-positive area to the whole plaque area was >40%. Plaque inflammation with macrophage and lymphocyte infiltration was recorded according to the number of CD68-negative or CD3-positive cells: infiltration of >20 inflammatory cells in the fibrous cap was defined as positive inflammation to the fibrous cap. Mural thrombus was defined as a fibrin organization of the endothelium or the fibrous cap of plaques (Fig 3, *C*).

Statistical analysis. Patients with mobile plaques and those with nonmobile plaques were compared for baseline characteristics, the prevalence of progressive symptoms, and plaque histologic features using a *t* test, the Wilcoxon rank sum test, or the Fisher exact test, as appropriate. Two-sided values of $P < .05$ were considered significant. Statistical analysis was performed with JMP 7.12 software (SAS Institute, Cary, NC).

RESULTS

Patients displaying mobile plaques and nonmobile plaques exhibited no significant difference in age, sex, diabetes mellitus, hyperlipidemia, smoking, coronary artery disease, administration of statins, administration of aspirin, or degree of stenosis (Table I). All statins were administrated with the usual doses (atorvastatin ≤20 mg, pravastatin ≤20 mg, or pitavastatin ≤2 mg), and no patients received high-dose statin therapy. Hypertension was observed more frequently in patients with nonmobile plaques.

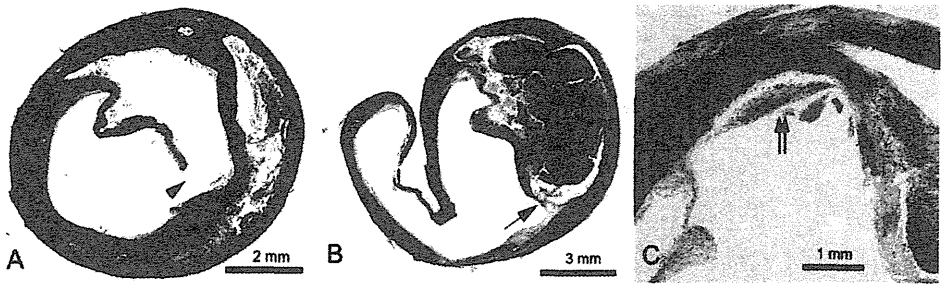

Fig 3. An example of histologic features of a mobile plaque (the same plaque as shown in Fig 1). **A**, A photomicrograph of the carotid bifurcation (Masson's trichrome staining, original magnification ×1) demonstrates complete disruption of the fibrous cap (*arrowhead*). **B**, Another cross-section (Masson's trichrome staining, original magnification ×1) demonstrates a large necrotic core with a fresh intraplaque hemorrhage (*asterisk*), which was covered with thin fibrous cap (*arrow*). **C**, A photomicrograph (Masson's trichrome staining, original magnification ×2) shows intramural fibrin deposit, indicating mural thrombus (*double arrow*).

Table I. Clinical characteristics at the time of carotid endarterectomy of study patients

Characteristics	*Mobile plaques (n = 18)*	*Nonmobile plaques (n = 17)*	P
Age, mean (SD), year	70.8 (11.5)	66.2 (8.7)	.19
Female, No. (%)	3 (16.7)	1 (5.9)	.60
Stenosis, mean (SD), %	73.2 (24.4)	76.5 (15.2)	.63
Risk factors, No. (%)			
Hypertension	11 (61.1)	17 (100)	.01
Diabetes mellitus	4 (22.2)	7 (41.2)	.29
Hyperlipidemia	11 (61.1)	12 (70.6)	.72
Smoking	9 (50.0)	13 (76.5)	.16
Medications, No. (%)			
Statin	7 (38.9)	7 (41.2)	1.00
Aspirin	10 (55.6)	13 (76.5)	.29
Interval to CEA, median (IQR) days			
From last ischemic event	12.5 (7.5-26.75)	33 (13.5-70.5)	.01
From last ultrasound study	3 (1-8.25)	9 (3.5-21.5)	.03
MRI-DWI positive, No. (%)	14 (77.8)	14 (82.4)	1.00

DWI, Diffusion-weighted image; *IQR,* interquartile range; *MRI,* magnetic resonance imaging; *SD,* standard deviation.

The median interval from the last ischemic event to CEA was 12.5 days (maximum, 41 days) in patients with mobile plaques, and the interval between the onset of symptoms and CEA, as well as that between ultrasound imaging and CEA, was significantly longer in patients with nonmobile plaques. No patients in this study had atrial fibrillation or other embolic sources. The incidence of the acute cerebral infarction detected with preoperative DWI did not show significant difference between mobile and nonmobile plaques (77.8% vs 82.4%, $P > .99$).

Clinical symptoms. The first ischemic symptoms among the 18 patients with mobile plaques were cerebral infarct in 11 patients, TSI in 5, TIA in 1, and amaurosis fugax without positive DWI finding in 1. Symptoms among 17 patients with nonmobile plaques included cerebral infarct in 6, TSI in 6, TIA in 3, and amaurosis fugax without positive DWI finding in 2. Progressive symptoms after admission were observed in six patients (33.3%) with mobile plaques, whereas no progression was seen in the patients with nonmobile plaques ($P = .02$).

Histologic features. All histologic features in mobile plaques and nonmobile plaques are summarized in Table II. The ratio of the mean cross-sectional area of the necrotic core to that of the entire plaque area was significantly larger in mobile plaques than in nonmobile plaques (mean, 0.660 vs 0.417, $P < .0001$).

Plaque ruptures were seen in 83% of mobile plaques. The median minimum cap thickness was 80 μm, which is smaller than that considered to be the critical value of minimum cap thickness for cap rupture.[16] There were no significant differences in the prevalence of cap rupture (83% vs 82%) or median minimum cap thickness (80 vs 100 μm) between mobile plaques and nonmobile plaques. The median area of the fibrous cap was, however, significantly smaller in the mobile plaques than in nonmobile plaques (9200 vs 15,900 μm^2; $P = .02$).

Although mural thrombus was more prevalent in mobile plaques (89%) than in nonmobile plaques (59%), the difference was not significant ($P = .060$). There was also no significant difference between mobile and nonmobile

Table II. Histologic features of mobile and nonmobile plaques

Feature	*Mobile plaques (n = 18)*	*Nonmobile plaques (n = 17)*	P
Fibrous cap			
Plaque rupture, No. (%)	15 (83)	14 (82)	>.99
Minimum cap thickness, median (IQR) μm	80 (60-162.5)	100 (60-250)	.33
Fibrous cap area, median (IQR) μm^2	9200 (6300-13,100)	15,900 (9000-21,800)	.02
Mural thrombus, No. (%)	16 (89)	9 (59)	.06
Ratio of necrotic core area, mean (SD)	0.660 (0.098)	0.417 (0.176)	<.0001
Inflammation of fibrous cap, No. (%)			
Macrophages	17 (94)	16 (94)	1.00
Lymphocytes	12 (67)	12 (71)	1.00
Intraplaque hemorrhage, No. (%)			
Fresh	11 (61)	9 (53)	.74
Previous	11 (61)	10 (59)	1.00

IQR, Interquartile range; *SD*, standard deviation.

plaques in the prevalence of cap inflammation or intraplaque hemorrhage.

DISCUSSION

One of our results showed that progressive symptoms occurred more frequently in patients with mobile plaques than those with nonmobile plaques. This result is in line with unstable neurologic presentations depicted in previous case reports of symptomatic mobile plaques.[5,8] A recent study also concluded that the jellyfish sign, which is an ultrasonographic appearance of mobile plaques, was an important predictive factor for repeated ischemic stroke.[12] These findings, along with our results, promoted us to confirm the hypothesis that some histologic differences may exist between mobile and nonmobile plaques, even if both are symptomatic plaques.

There are several reports about mobile plaques in large arteries, including the carotid artery,[2-8,19,20] and some reports have described the histology of mobile plaques. Nakajima et al[5] reported the pathologic findings of a mobile plaque in the brachiocephalic artery that caused fatal recurrent strokes and pointed out that plaque disruption was a cause of the mobility. Arning et al[6] also found a mural thrombus in the histology of a mobile carotid plaque. Our results are compatible with previous pathologic reports about mobile plaques. However, the features of plaque rupture and mural thrombus may not be sufficient to describe the specific histopathology of a mobile plaque, because according to our result, the prevalence of plaque disruption or mural thrombus of mobile plaques is not significantly higher than that of nonmobile plaques. The findings of the present study suggest that the existence of a large, soft, lipid-rich necrotic core is also important for the mechanism of plaque mobility.

This speculation conforms with the results on intravascular ultrasound elastography, a recently developed technique to assess the elasticity of plaque tissue using intravascular ultrasound imaging by measuring the "strain" or small movement of plaque tissue under an applied force.[21,22] The authors of those studies demonstrated that the strain could distinguish lipid-rich components from hard and fibrous components. Mobile structures on carotid plaques may be caused not only by the mural thrombus formed by a fibrous cap rupture but also by a large, lipid-rich necrotic core exposed into the blood lumen.

Several pathologic reviews have reinforced the evidence that a large necrotic core is one of the important features of so-called vulnerable plaque.[23-27] Studies on aortic plaques,[28,29] an in vivo study on magnetic resonance imaging,[30] and histologic studies of carotid plaque[16,31] have also shown that a large necrotic core was strongly associated with thrombosis, fibrous cap rupture, fibrous cap thinning, or neurologic symptoms. Moreover, when the large lipid-rich necrotic core is exposed to blood lumen by plaque rupture, the mural thrombus or debris from the lipid core may become a persistent source of embolism, which may result in an unstable ischemic stroke. On the other hand, Redgrave et al[16] showed that thinning of the fibrous cap covering the necrotic core is another important factor for plaque vulnerability. These authors advocated critical cap thickness (minimum cap thickness <200 μm and a representative cap thickness <500 μm) as a marker for ruptured plaque.

Using these criteria, the fibrous cap of both groups in our study is extremely thin. One possible reason that we did not find a significant difference in minimum fibrous cap thickness in the two plaques is that mobile and nonmobile plaques had an almost equally high prevalence of cap rupture. Given that the actual fibrous cap area was smaller in mobile plaques than in nonmobile plaques (Table II), it is possible that the overall thickness of fibrous caps in mobile plaques is smaller than that in nonmobile plaques.

To our knowledge, only one recent study demonstrated the histologic features of mobile carotid plaques, although it did not include a controlled group in histologic assessment. Kume et al[12] examined histologic features of 15 plaques with ultrasonographic jellyfish sign, and the results showed that the proportional area of the fibrous cap correlated negatively with jellyfish-positive plaque surface movement rate. Our results regarding the fibrous cap area

coincides with their results. They could not, however, document significant correlation between atheromatous lesion area and the plaque motion rate, which is inconsistent with our results. This may be attributed to the differences in a patient population, in definitions of ultrasonographic and histopathologic findings, and in research designs between the two studies. The large necrotic core can still be one of the representative features of mobile plaques because the present results were conducted in a controlled study.

Our study has some limitations. First, the number of the cases we studied was small, and not all of the nonmobile plaques were examined. Larger studies are necessary to confirm higher prevalence of mural thrombus and thinner fibrous cap in mobile plaques.

Second, because nonmobile plaques had a longer duration until CEA, stabilization of the plaque, which can occur within approximately 90 days after the presenting neurologic symptom,[31,32] could have led us to underestimate some of the histologic factors of nonmobile plaques. However, one study showed that the prevalence of a large lipid core and mural thrombus was not influenced by the span of time since the last ischemic events.[31] Spagnoli et al[33] also revealed that a fresh thrombus can present several months after the first cerebrovascular event.

Third, we only studied symptomatic plaques and did not include asymptomatic plaques. Our study, therefore, did not answer the question of whether mobile plaques are more "vulnerable" than nonmobile plaques as long as the word "vulnerable" means the tendency for fibrous cap rupture and the potential of subsequent embolic stroke. That was not, however, the purpose of this study. Further studies including both symptomatic and asymptomatic patients might be essential to determining whether mobile plaques are more "vulnerable" than nonmobile plaques.

A higher prevalence of mobile plaques is shown in this report (9.3% of excised plaque) than in previous study.[1] The most recent study demonstrated an even higher prevalence (19%) of mobile plaques.[12] This high prevalence of mobile plaques may be explained by the evolution of the duplex ultrasound imaging system and sheds light on the clinical importance of mobile plaques.

CONCLUSIONS

In this study, we have clarified the histologic difference between mobile and nonmobile symptomatic carotid plaques. This result may partly explain unstable neurologic presentations of patients with mobile carotid plaques and may add information to the debate regarding the management of mobile plaques. Further studies on the histopathology and natural history of the mobile carotid plaque are needed to establish the most effective acute management for symptomatic mobile plaques.

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AUTHOR CONTRIBUTIONS

Conception and design: TF, KI
Analysis and interpretation: TF, KN, HI
Data collection: TF, KN, TH
Writing the article: TF
Critical revision of the article: KI, SM
Final approval of the article: KI, HI
Statistical analysis: TF
Obtained funding: Not applicable
Overall responsibility: TF

REFERENCES

1. Szendro G, Sabetai MM, Tegos TJ, Dhanjil S, Lennox AF, Nicolaides AN. Mobile carotid plaques: the natural history of two asymptomatic and non-operated cases. J Vasc Surg 1999;30:357-62.
2. Cho YP, Kwon TW, Kim GE. Sonographic appearance of a free-floating atheromatous plaque in a patient with acute stroke. J Clin Ultrasound 2002;30:317-21.
3. Ferrero E, Gaggiano A, Ferri M, Nessi F. Mobile floating carotid plaque post-trauma. Diagnosis and treatment. Interact Cardiovasc Thorac Surg 2009;8:496-7.
4. Ko PT, Lin SK, Chang YJ, Ryu SJ, Chu CC. Carotid floating plaques associated with multiple cerebral embolic strokes. Case reports. Angiology 1997;48:255-61.
5. Nakajima M, Yasaka M, Minematsu K. Mobile thrombus from a ruptured plaque in the brachiocephalic artery. J Stroke Cerebrovasc Dis 2008;17:423-5.
6. Arning C, Herrmann HD. Floating thrombus in the internal carotid artery disclosed by B-mode ultrasonography. J Neurol 1988;235:425-7.
7. Combe J, Poinsard P, Besancenot J, Camelot G, Cattin F, Bonneville JF, et al. Free-floating thrombus of the extracranial internal carotid artery. Ann Vasc Surg 1990;4:558-62.
8. Choukroun EM, Labrousse LM, Madonna FP, Deville C. Mobile thrombus of the thoracic aorta: diagnosis and treatment in 9 cases. Ann Vasc Surg 2002;16:714-22.
9. Di Tullio MR, Russo C, Jin Z, Sacco RL, Mohr JP, Homma S. Aortic arch plaques and risk of recurrent stroke and death. Circulation 2009; 119:2376-82.
10. Ay H, Koroshetz WJ, Benner T, Vangel MG, Wu O, Schwamm LH, et al. Transient ischemic attack with infarction: a unique syndrome? Ann Neurol 2005;57:679-86.
11. Fox AJ. How to measure carotid stenosis. Radiology 1993;186:316-8.
12. Kume S, Hama S, Yamane K, Wada S, Nishida T, Kurisu K. Vulnerable carotid arterial plaque causing repeated ischemic stroke can be detected with B-mode ultrasonography as a mobile component: jellyfish sign. Neurosurg Rev 2010;33:419-30.
13. Alers JC, Krijtenburg PJ, Vissers KJ, van Dekken H. Effect of bone decalcification procedures on DNA in situ hybridization and comparative genomic hybridization. EDTA is highly preferable to a routinely used acid decalcifier. J Histochem Cytochem 1999;47:703-10.
14. Bassiouny HS, Davis H, Massawa N, Gewertz BL, Glagov S, Zarins CK. Critical carotid stenoses: morphologic and chemical similarity between symptomatic and asymptomatic plaques. J Vasc Surg 1989;9:202-12.
15. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003;349:2316-25.
16. Redgrave JN, Gallagher P, Lovett JK, Rothwell PM. Critical cap thickness and rupture in symptomatic carotid plaques: the Oxford plaque study. Stroke 2008;39:1722-9.
17. Lovett JK, Gallagher PJ, Rothwell PM. Reproducibility of histological assessment of carotid plaque: implications for studies of carotid imaging. Cerebrovasc Dis 2004;18:117-23.
18. Bassiouny HS, Sakaguchi Y, Mikucki SA, McKinsey JF, Piano G, Gewertz BL, et al. Juxtalumenal location of plaque necrosis and neo-