Transcriptase-Polymerase Chain Reaction (PT-PCR) を行った．Primer sequencesは，表1に示すとおりである．

2.3 COX-2のimmunoblot analysis

SD誘発側皮質（右側大脳皮質）におけるCOX-2蛋白の発現を，以前報告した方法に従って[8]，immunoblot analysisにて分析した．各群におけるCOX-2蛋白の発現は，コントロールの動物での発現に対する比で表した．

2.4 プロスタグランジン生成物の測定

両側大脳半球，脳組織におけるプロスタグランジンE_2濃度を，radioimmunoassay (PerkinElmer Life Sciensea, Inc. MA, USA) にて測定した．

3. 成　　績

実験中，各群間における体温，動脈血ガス分析値に有意差はなかった．SD誘発回数は，I群では0回，II群では4.7±2.4回（平均値±SD），III群では3.6±1.6回であり，II群とIII群には，有意差はなかった．

3.1 SD関連遺伝子

II群における遺伝子発現が，I群及びIII群，いずれの群に対しても2.5倍以上増えていた遺伝子に，S-100A9遺伝子とmitogen-activated protein kinase phosphatase (cpg21) があった．これらの遺伝子とCOX-2遺伝子発現をRT-PCRを用いて分析した．I群における，これら三つの遺伝子発現は，両側大脳皮質において有意差はなかった．すべての遺伝子は，I群に比較してII群のSD誘発側（右側）皮質でup-regulateされていた（図2）．II群のSD誘発（右）側においては，COX-2及びcpg21 mRNAどちらの発現も，SD非誘発（左）側に比較して，有意にup-regulateされていた．他方，SD非誘発（左）側でのS-100A9 mRNAの発現は，右側での発現と同程度であった．III群での両側におけるS-100A9 mRNAの発現は，いずれもII群に比較して有意に低下していた．

3.2 COX-2蛋白発現とPGE_2合成

II群及びIII群のSD誘発（右）側におけるCOX-2蛋白の平均発現比は，それぞれ1.58（II群），1.80（III群）であり，I群に比較して有意に増加していた（図3）．III群の両側大脳半球における組織PGE_2の平均濃度は，コントロール（I）群に比較して有意に低かった（表2）．III群のSD誘発（右）側におけるPGE_2濃度は，対側に比べて有意に低かった．

4. 考　　察

今回我々は，選択的COX-2阻害薬JTE-522が，SD誘発脳での遺伝子発現に対して，どの様な影響を及ぼすかについて検討した．COX-2発現がSD誘発側でのみup-egulateされたのは，Choudhuri[14]らの報告と一致した結果であった．II及びIII群での右側皮質におけるCOX-2 mRNAとCOX-2蛋白の発現は，I群での発現に比べてそれぞれ7～9倍と約2倍であり，両者の発現には解離が見られた．一方PGE_2合成は，I，II群で同様であったが，

表1　Oligonucleotide primers used in RT-PCR

Gene	Sequences (Forward primer) (Reverse primer)	Cycling Number(N) PCR Product size
COX-2	CCCAGCACTTCACTCATCAGTTTTTCAAGA(F563)	32
	TTCCACCAGCAGGGCGGGATACAGTTCCAT(R1459)	926bp
cpg21	GAGTATATCAAGCAGAGGAGGAGCGTGGTC(1045F)	32
	TTCCCTGAAGTGACAGAGGACAGAGACAGA(1761R)	746bp
S-100A9	AGCGCAGCATAAGCACCATCATCAATGTTT(60F)	32
	ATTATTTCCCAGCCCCAGAACCAAGGTCAT(431R)	401bp
GAPDH	ACCACAGTCCATGCCATCAC(586F)	23
	TCCACCACCCTGTTGCTGTA(1018R)	439bp

COX-2, cyclooxygenase 2 ; cpg21, mitogen-activated protein kinase ; GAPDH, glyceraldehydes-3-phosphate dehydrogenase

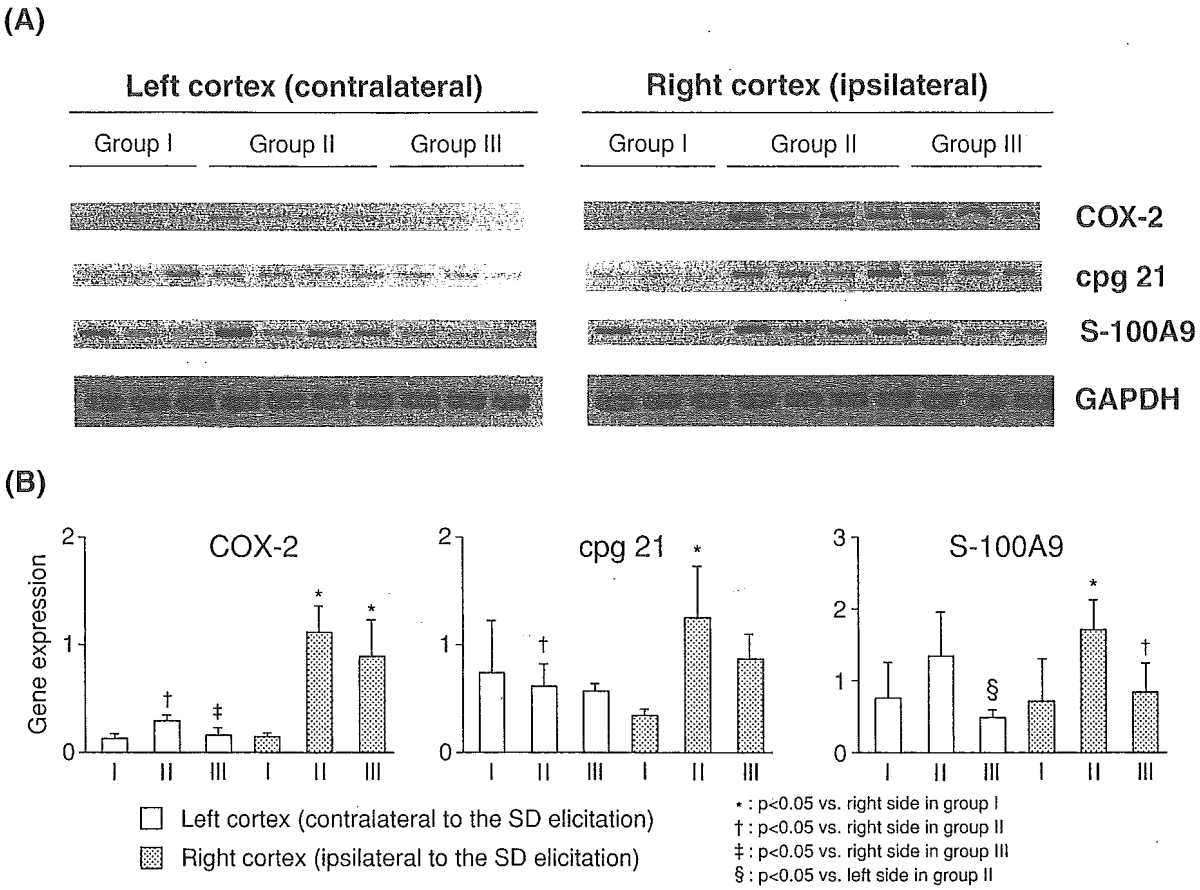

図2 RT-PCR analysis of COX-2, cpg21, S-100A9, and GAPDH mRNA in each group.
(A) Autoradiograms of COX-2, cpg21, S-100A9, and GAPDH mRNA in each group.
(B) Densitometric analysis showing the expression of the genes of interest normalized with GAPDH mRNA. Note that S-100A9, which was upregulated in both cortices of rats in group II, was downregulated by the administration of JTE-522 (group III). The expression of COX-2 and cpg 21 was prominent in the cortices undergoing SD in the rats in group II. Expression of GAPDH mRNA was equivalent between groups.

III群の右側皮質では，対側に比較して有意に低かった．こうした所見は，複数回のSD誘発による蛋白合成の抑制[15]，若しくは，COX-2酵素の速い代謝分解速度[16,17]に起因していると考えられた．

cpg21は，ヒトにおけるdual specificity phosphatase 5と92％の相同性を有し，phosphorylated extracellular signal regulated kinase 1 (ERK 1) の脱リン酸化と不活性化に関与する．MAP kinase/ERK kinase (MEK) に依存した，SD発生にひき続いて生じる一過性のERK1/2のリン酸化レベルは，SD発生45分後にコントロールレベルに戻ると報告されている[18]．我々は，RT-PCRによってJTE-522は，cpg21遺伝子発現を抑制しなかったことを確認した．

S-100A9は，カルシウム結合蛋白であるS-100ファミリーに属している．S-100蛋白は，1965年，Mooreによって[19]初めて分離され，次の三つの二量体の型がある：α-α蛋白のS-100A (0)，α-β蛋白のS-100Aとβ-β蛋白のS-100Bである[20]．脳梗塞巣周囲のreactive astrocyteによるS-100B蛋白合成の増加は，ラット局所脳虚血における遅発性梗塞巣拡大に関連した炎症反応に加担することが示された[21]．S-100A8とS-100A9は，活性化好中球や単球によって合成され，細胞膜に移動し，細胞骨格蛋白とともにヘテロダイマーを形成する[22,23]．Postlerらは[24]，剖検脳より，虚血早期にS-100A8とS-100A9が梗塞巣周囲のmicroglia cellsに発現していると報告したが，それらの働きについては，

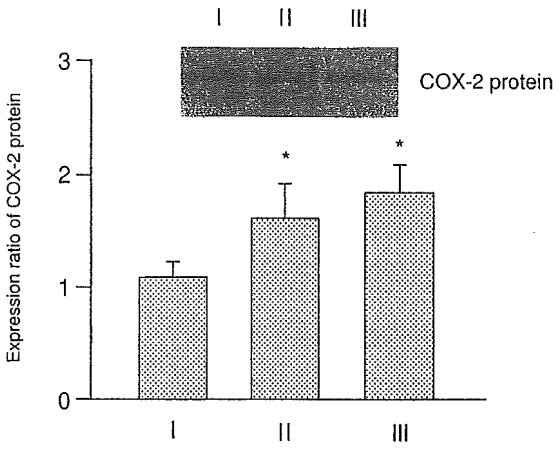

図 3 Expression of COX-2 protein in the right cortex. Immunoblot analysis revealed a 70- to 72-kd COX-2-immunoreactive band. The mean expression ratio of COX-2 protein in groups II and III was significantly higher than that seen in group I (* $p<0.05$).

表 2 Concentration of PGE_2 in brain samples

Group	Right cortex (ipsilateral)	Left cortex (contralateral)
I	22.8±12.3	36.3±16.9
II	17.8±12.6	29.5±13.8
III	8.0± 4.9*‡	18.4± 6.9†

*: $p<0.05$ vs. right cortex in group I,
†: $p<0.05$ vs. left cortex in group I,
‡: $p<0.05$ vs. left cortex in group III
pg/TP(total protein) mg

明らかではない．

今回我々は，S-100A9 遺伝子発現が SD 誘発側皮質のみならず，対側にも発現していることを明らかにした．S-100A8/A9 複合体は，カルシウム濃度依存性に，多価不飽和脂肪酸と特異的に結合し[25,26]，アラキドン酸代謝酵素活性を修飾するという[27]．SD による神経活性は，N-methyl-D-aspartate (NMDA) 受容体を介した astrocyte を伝搬するカルシウム波によって，遠隔領域に伝わりうると考えられた[28-30]．S-100A9 遺伝子発現と PGE_2 合成は，JTE-522 投与によって両側性に down-regulate されたことより，S-100A9 遺伝子発現は，SD によるカルシウム濃度変化のみならず COX-2 活性の増加によって影響されると考えられた．

5. 結　論

SD 関連遺伝子である S100A9 遺伝子は，プロスタグランジン生成により発現調節されていると考えられた．

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C-Reactive Protein and Risk of First-Ever Ischemic and Hemorrhagic Stroke in a General Japanese Population

The Hisayama Study

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Background and Purpose—The role of high-sensitivity C-reactive protein (hsCRP) in the development of stroke is not clearly understood. We investigated the relationship between serum hsCRP levels and stroke occurrence in a general Japanese population.

Methods—We followed 2692 subjects ≥40 years of age for 12 years. The relative risks and 95% CIs for ischemic and hemorrhagic stroke occurrence were calculated according to the hsCRP quintiles.

Results—During the follow-up, 129 first-ever ischemic and 59 hemorrhagic strokes occurred. In men, the age-adjusted incidence of ischemic stroke significantly increased with elevated serum hsCRP levels; the difference between the first and fifth quintiles was statistically significant (1.4 versus 6.6 per 1000 person-years; $P=0.02$). This association remained significant even after adjustment for other confounding factors, such as age, systolic blood pressure, ECG abnormalities, diabetes, body mass index, total cholesterol, high-density lipoprotein cholesterol, smoking habits, alcohol intake, and regular exercise (adjusted relative risks, 3.11; 95% CI, 1.04 to 9.32; $P=0.04$). However, such associations were not observed for ischemic stroke in women or in hemorrhagic stroke in either sex. Among male subjects who were both in the fifth hsCRP level and had hypertension, diabetes, obesity, hypercholesterolemia, or a smoking habit, the risk of ischemic stroke was extremely increased, even after adjustment for other risk factors.

Conclusions—Our findings suggest that elevated serum hsCRP levels are an independent risk factor for future ischemic stroke in Japanese men and that the coexistence of a high hsCRP level with another risk factor extremely increases the risk of ischemic stroke. (***Stroke.*** **2006;37:27-32.)**

Key Words: C-reactive protein ■ hemorrhage, brain ■ ischemic stroke

C-reactive protein (CRP), an acute-phase reactant, increases significantly in inflammatory disorders[1] and enhances immune reactivity.[2] Recently, the role of endothelial cells and monocytes in the inflammatory process has become better understood,[3] and inflammation has emerged as an important factor in atherosclerosis. Consequently, high-sensitivity CRP (hsCRP) levels have attracted clinical attention as a predictive marker of atherosclerosis. Several epidemiological studies have reported that hsCRP levels were positively associated with the risk of cardiovascular disease.[4–9] Most of those studies examined coronary heart disease[4–6] or combined end points of coronary heart disease and ischemic stroke,[7–9] whereas only a few studies examined ischemic stroke.[10–12] The subjects of the latter studies were limited to the elderly[10,11] or men,[12] and we found no studies on hemorrhagic stroke.

The purpose of the present study was to examine the relationship between serum hsCRP levels and the development of ischemic and hemorrhagic stroke in a prospective study of a general population consisting of middle-aged and elderly Japanese men and women.

Methods

Study Population

Since 1961, we have been conducting a long-term prospective cohort study of cardiovascular disease in the town of Hisayama, a suburb of Fukuoka City in Southern Japan. In 1988, a screening survey for the present study was performed in the town.[13] A total of 2742 residents ≥40 years of age (80.9% of the total population of this age group) consented to participate in the examination. After excluding 96 subjects with a history of stroke or myocardial infarction and 54

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subjects whose frozen blood samples were insufficient for the measurement of serum hsCRP, the remaining 2592 individuals were enrolled in this study.

Follow-Up Survey

This population was followed up for 12 years, from December 1988 through November 2000, by repeated health examinations or by a daily monitoring system established by the study team and local physicians or members of the Health and Welfare Office for the town. A detailed description of the study methods was published previously.[14,15]

During the follow-up period, 188 subjects were moved out of town, and only 1 subject declined to be followed up. For subjects who did not undergo regular examinations or who moved out of town, their health status was checked by mail or telephone once a year. When new neurological symptoms were suspected, study-team physicians evaluated the subject's detailed diagnostic information. The clinical diagnosis of stroke was based on the detailed history, neurological examinations, and ancillary laboratory examinations.

Stroke Classification

Stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for >24 hours and was classified as either ischemic or hemorrhagic (cerebral hemorrhage or subarachnoid hemorrhage). Rare causes of cerebrovascular disease, such as collagen disease, hematologic disorder, trauma, chronic subdural hematoma, or moyamoya disease, were not considered in stroke cases. The diagnosis and classification of stroke were based on clinical information, ancillary laboratory examinations (such as brain imaging including computed tomography and MRI, cerebral angiography, echocardiography, and carotid duplex imaging), and autopsy findings.

During the follow-up period, 188 subjects developed first-ever stroke. During the follow-up, 92 of the 188 first-stroke cases died, and, of these, 71 (77.2%) underwent autopsy examination. The first-stroke cases were classified as 129 ischemic strokes (56 men and 73 women) and 59 hemorrhagic strokes (25 men and 34 women).

Risk Factors

Plasma glucose levels were determined by the glucose-oxidase method, and diabetes mellitus was defined by a 75-g oral glucose tolerance test and by fasting (≥7.0 mmol/L) or postprandial blood glucose level (≥11.1 mmol/L) or by the use of hypoglycemic agents. Total cholesterol and high-density lipoprotein (HDL) cholesterol levels were determined enzymatically. Hypercholesterolemia was defined as a serum cholesterol level of ≥5.69 mmol/L. Serum specimens collected at the time of CRP measurement were stored at −20°C until they were used in 2002. Serum hsCRP levels were analyzed using a modification of the Behring latex-enhanced CRP assay on a Behring nephelometer BN-100 with a 2% interassay coefficient of variation.

Sitting blood pressure was measured 3 times at the right upper arm using a sphygmomanometer after ≥5 minutes of rest; the average of the 3 measurements was used in the analysis. Hypertension was defined as systolic blood pressure of ≥140 mm Hg and diastolic blood pressure of ≥90 mm Hg and current treatment with antihypertensive agents. Height and weight were measured in light clothes without shoes, and the body mass index (BMI, kg/m^2) was calculated. Obesity was defined as a BMI of ≥25 kg/m^2. ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code,[16] 3-1) and ST depression (4-1,2,3) and atrial fibrillation (8-3).

Information on smoking habits, alcohol intake, and physical activity during leisure time was obtained with the use of a standard questionnaire. Smoking habits and alcohol intake were classified as either current or not. Those subjects engaging in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group.

Statistical Analysis

In both men and women combined, we found a significant interaction between sex and hsCRP levels on the risk of ischemic stroke, so the additional analyses were performed separately for men and women by using sex-specific quintiles of hsCRP: Q1, 0.05 to 0.20; Q2, 0.21 to 0.40; Q3, 0.41 to 0.71; Q4, 0.72 to 1.56; and Q5, 1.57 to 14.20 mg/L for men and 0.05 to 0.17, 0.18 to 0.30, 0.31 to 0.53, 0.54 to 1.09, and 1.10 to 13.00 mg/L, respectively, for women. The incidence rates were calculated by the person-year method and adjusted for age by the direct method using 10-year age groupings. The multivariate-adjusted relative risks (RRs) and 95% CIs were calculated according to the hsCRP quintile distribution, using the stepwise Cox proportional hazards model with $P<0.2$ required for entering or remaining in the model. The interaction between 2 risk factors on the risk of stroke was tested by the χ^2 test. A $P<0.05$ was considered to indicate statistical significance.

Results

The baseline characteristics of the subjects are shown in Table 1. The mean age was 58 years for men and 59 years for women. Compared with women, men had higher mean levels of serum hsCRP and systolic and diastolic blood pressures, as well as higher frequencies of hypertension, ECG abnormalities, diabetes mellitus, current smoking, current drinking, and regular exercise, whereas women had higher mean levels of BMI, total cholesterol, and HDL cholesterol.

Figure 1 shows the age-adjusted incidence rates of first-ever ischemic stroke according to quintiles of baseline serum hsCRP. The incidence rates of ischemic stroke were 1.4, 1.9, 5.8, 4.2, and 6.6 per 1000 person-years from the first to fifth quintiles of hsCRP for men and 2.0, 3.4, 5.4, 2.9, and 2.7 per 1000 person-years, respectively, for women. In men, the incidence of stroke rose significantly with rising serum hsCRP levels ($P<0.01$ for trend), and the incidence for subjects in the fifth quintile was ÷5-fold that of subjects in the first quintile ($P=0.02$). However, such an association was not seen in women ($P=0.71$ for trend). On the other hand, the age-adjusted incidence rates of first-ever hemorrhagic stroke were 2.4, 1.1, 2.2, 1.9, and 2.7 per 1000 person-years, respectively, for men, and 1.1, 2.6, 1.0, 1.3, and 1.6 per 1000 person-years, respectively, for women, and there were no significant trends in either sex (Figure 2).

TABLE 1. Baseline Characteristics of Study Subjects, the Hisayama Study, 1988

Characteristic	Men (n=1092)	Women (n=1500)
Age, y	58.1±11.4	59.4±11.9
High-sensitivity C-reactive protein, mg/L		
Median	0.54	0.40
Mean	2.07±8.31	1.30±5.45
Systolic blood pressure, mm Hg	134.7±20.1	132.9±22.2
Diastolic blood pressure, mm Hg	80.5±11.4	75.8±10.8
Hypertension, %	45.2%	38.5%
Use of antihypertensive agents, %	14.2%	15.4%
ECG abnormalities, %	20.7%	14.7%
Diabetes mellitus, %	15.1%	9.6%
BMI, kg/m^2	22.8±2.9	22.9±3.3
Total cholesterol, mmol/L	5.09±1.07	5.54±1.07
HDL-cholesterol, mmol/L	1.25±0.31	1.33±0.30
Current smoking, %	49.8%	6.7%
Current drinking, %	60.6%	9.0%
Regular exercise, %	11.8%	9.1%

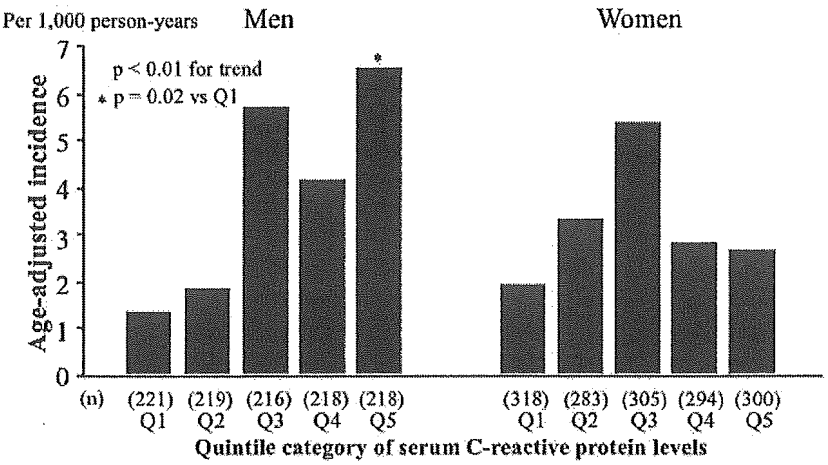
Data are mean±1 SD or percent, unless otherwise specified.

Table 2 shows the multivariate-adjusted RRs and their 95% CIs for the development of ischemic and hemorrhagic stroke according to hsCRP quintile categories. In men, the risk of ischemic stroke significantly increased with rising hsCRP levels even after adjustment for age, systolic blood pressure, ECG abnormalities, diabetes, BMI, total cholesterol, HDL cholesterol, smoking habits, alcohol intake, and physical activity (P=0.02 for trend), and the multivariate-adjusted RR of subjects in the fifth quintile was significantly higher than that of subjects in the first quintile (RR, 3.11; 95%CI, 1.04 to 9.32; P=0.04). However, such associations were not observed for ischemic stroke in women or for hemorrhagic stroke in either sex (Table 2). To examine the combined effects of elevated hsCRP levels and other cardiovascular risk factors on ischemic stroke occurrence, we estimated the age-adjusted RRs of ischemic stroke among 4 groups of male subjects according to the presence or absence of a high-hsCRP level (the fifth quintile, ≥1.57 mg/L) and each risk factor (Table 3). Compared with the reference group having neither high-hsCRP levels nor hypertension, the risk of ischemic stroke for the groups with either high-hsCRP levels or hypertension was not significant, but the risk for the group having both high-hsCRP levels and hypertension was significantly higher (RR, 2.77; 95% CI, 1.31 to 5.83; P<0.01). A similar pattern was observed for the coexistence of high-hsCRP levels and diabetes (RR, 4.30; 95% CI, 1.89 to 9.79; P<0.01), obesity (RR, 4.00; 95% CI, 1.53 to 10.46; P< 0.01), hypercholesterolemia (RR, 3.74; 95% CI, 1.71 to 8.19; P<0.01), or smoking habits (RR, 2.29; 95% CI, 1.78 to 4.87; P=0.03). There were significant interactions between high-hsCRP levels and diabetes (χ^2=5.370; P=0.02), as well as hypercholesterolemia (χ^2=6.052; P=0.01), and a marginally significant interaction (χ^2=3.39; P=0.06) between high-hsCRP levels and hypertension. However, interactions for obesity and smoking were not significant. These associations were substantially unchanged even after adjustment for other risk factors in the multivariate analysis.

Discussion

In a 12-year follow-up examination of a general Japanese population, we demonstrated that elevation of serum hsCRP levels was an independent risk factor for future ischemic stroke in men but not in women, whereas there was no association between serum hsCRP levels and the risk of future hemorrhagic stroke in either sex. Moreover, the coexistence of a high-hsCRP level and another risk factor, such as hypertension, obesity, diabetes, hypercholesterolemia, or smoking, extremely increased the risk of future ischemic stroke in our male subjects.

Recently, the Framingham Study[10] and Cardiovascular Health Study,[11] both which had elderly subjects (mean age,



Quintile range (mg/l)
Men:Q1= ≤ 0.20, Q2=0.21 to 0.40, Q3=0.41 to 0.71, Q4=0.72 to 1.56, Q5=1.57
Women:Q1= ≤ 0.17, Q2=0.18 to 0.30, Q3=0.31 to 0.53, Q4=0.54 to 1.09, Q5=1.10

Figure 1. Age-adjusted incidence rates of first-ever ischemic stroke according to serum high-sensitivity C-reactive protein levels.

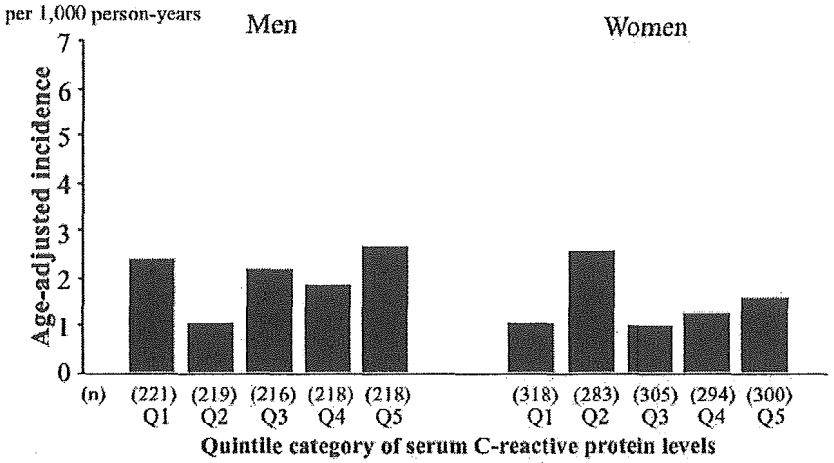


Figure 2. Age-adjusted incidence rates of first-ever hemorrhagic stroke according to serum high-sensitivity C-reactive protein levels.

Quintile range (mg/l)
Men:Q1= ≤ 0.20, Q2=0.21 to 0.40, Q3=0.41 to 0.71, Q4=0.72 to 1.56, Q5=1.57
Women:Q1= ≤ 0.17, Q2=0.18 to 0.30, Q3=0.31 to 0.53, Q4=0.54 to 1.09, Q5=1.10

69.8 and 72.6 years, respectively), and a nested case-control study of Japanese-American men[12] in Hawaii have investigated the association between hsCRP level and the risk of future ischemic stroke. In those studies, the elevation of serum hsCRP was clearly associated with ischemic stroke in men, which support our findings. For women, on the other hand, the effects of high levels of serum hsCRP on ischemic stroke were ambiguous. In the Framingham study women, hsCRP levels were significantly associated with the risk of ischemic stroke,[10] whereas no significant association was observed for the women in the Cardiovascular Health Study,[11] which was in accord with the findings of our study. Recent clinical evidence has shown that endogenous estrogen protects the development of atherosclerosis[17,18] and that estrogen induces the elevation of hsCRP levels.[19] In women, such conflicting effects of sex hormone might weaken the association of hsCRP elevation with ischemic stroke. Another reason for the sex difference in the risk of ischemic stroke might stem from the difference in the atherosclerotic process between men and women. Generally, it is considered that atherosclerosis is more severe in men than in women. Thus, it may be easier to detect the association between hsCRP levels and ischemic stroke in men.

TABLE 2. Multivariate-Adjusted RRs of First-Ever Ischemic and Hemorrhagic Stroke according to Serum High-Sensitivity C-Reactive Protein Levels

Quintiles of Men/Women	RR	95% CI	*P* Value	RR	95% CI	*P* Value
Ischemic stroke						
Q1	1.00	1.00				
Q2	1.08	0.29 to 4.03	0.91	1.27	0.55 to 2.94	0.58
Q3	2.81	0.93 to 8.51	0.07	1.56	0.71 to 3.39	0.27
Q4	2.24	0.73 to 6.92	0.16	1.05	0.46 to 2.42	0.90
Q5	3.11	1.04 to 9.32	0.04	1.34	0.61 to 2.91	0.46
P for trend	0.02	0.65				
Hemorrhagic stroke						
Q1	1.00			1.00		
Q2	0.33	0.07 to 1.65	0.18	2.66	0.82 to 8.61	0.10
Q3	0.58	0.17 to 1.91	0.37	1.00	0.24 to 4.06	0.99
Q4	0.78	0.26 to 2.37	0.67	2.10	0.63 to 7.04	0.23
Q5	0.68	0.21 to 2.26	0.53	1.74	0.51 to 5.85	0.37
P for trend	0.92	0.64				

Men, mg/L: Q1=≤0.20, Q2=0.21 to 0.40, Q3=0.41 to 0.71, Q4=0.72 to 1.56, Q5=≥1.57. Women, mg/L: Q1=≤0.17, Q2=0.18 to 0.30, Q3=0.31 to 0.53, Q4=0.54 to 1.09, Q5=≥1.10. Multivariate adjustment was made for age, systolic blood pressure, ECG abnormalities, diabetes, BMI, total cholesterol, HDL cholesterol, smoking habits, alcohol intake, and physical activity.

In our subjects, we did not find a clear association between hsCRP levels and hemorrhagic stroke occurrence. Because cerebral hemorrhage develops from the rupture of small vessels, such as cerebral perforating arteries, damaged by hypertension causing lipohyalinosis,[20] or by amyloid angiopathy,[21] it is suggested that elevated hsCRP levels have little or no association with small vessel disease. Although hypertension and smoking may accelerate the development and growth of intracranial aneurysm,[22] which is a main cause of subarachnoid hemorrhage, the association between atherosclerosis and intracranial aneurysm is considered weak.[23] Thus, our finding that there is no association between serum hsCRP levels and hemorrhagic stroke is reasonable.

Our stratified analysis showed an extremely increased risk of ischemic stroke in men who have both a high-hsCRP level and another risk factor. Although the mechanism underlying this phenomenon is not clearly understood, several possible explanations have been proposed. Because inflammation is strongly related to atherosclerosis, elevated hsCRP levels may reflect the existence of advanced atherosclerosis induced by other cardiovascular risk factors. Accordingly, it is conceivable that the coexistence of elevated hsCRP levels and other risk factors is a marker of a group at high risk of atherosclerosis, and, thus, the risk of ischemic stroke is considerably high in that group. Additionally, recent clinical

TABLE 3. Age-Adjusted RRs of First-Ever Ischemic Stroke according to High-Sensitivity C-Reactive Protein Levels and Risk Factors in Men

Risk Factor	CRP Levels	Events/Populations (n)	RR	95% CI	*P* Value
Hypertension					
No	Low	16/472	1.00		
Yes	Low	22/363	1.34	0.69 to 2.56	0.39
No	High	5/105	1.27	0.46 to 3.47	0.65
Yes	High	13/96	2.77	1.31 to 5.83	<0.01
Diabetes mellitus					
No	Low	30/719	1.00		
Yes	Low	8/116	1.65	0.75 to 3.59	0.21
No	High	11/167	1.42	0.71 to 2.84	0.32
Yes	High	7/34	4.30	1.89 to 9.79	<0.01
Obesity					
No	Low	47/635	1.00		
Yes	Low	11/200	1.91	0.93 to 3.93	0.08
No	High	13/162	1.69	0.87 to 3.29	0.12
Yes	High	5/39	4.00	1.53 to 10.46	<0.01
Hypercholesterolemia					
No	Low	31/617	1.00		
Yes	Low	7/218	0.77	0.34 to 1.75	0.54
No	High	10/145	1.15	0.56 to 2.35	0.71
Yes	High	5/56	3.74	1.71 to 8.19	<0.01
Current smoking					
No	Low	21/432	1.00		
Yes	Low	17/403	1.11	0.59 to 2.12	0.74
No	High	8/87	1.48	0.65 to 3.36	0.35
Yes	High	10/114	2.29	1.78 to 4.87	0.03

CRP levels: "high" indicates the fifth quintile; low, the first to fourth quintiles. Hypertension: systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive agents. Diabetes: fasting blood glucose ≥7.0 mmol/L, or postprandial blood glucose level ≥11.1 mmol/L, or current use of hypoglycemic agents. Obesity: BMI ≥25 kg/m^2. Hypercholesterolemia: total cholesterol level ≥5.69 mmol/L.

reviews, as well as experimental and clinical studies, have shown that inflammation is directly associated with the development of atherosclerosis[24] and instability of atheroma.[25,26] It is, therefore, speculated that chronic inflammation directly and extremely enhances the risk of ischemic stroke by such atherogenic effects of inflammation in people whose arterial walls have already been damaged by other risk factors.

Several limitations of our study should be discussed. The primary limitation is that our findings are based on a 1-time measurement of serum hsCRP, which may not accurately reflect the status of the study participants. However, this source of variability could not account for the relationship observed in the present study, because a random misclassification of such nature would tend to underestimate study findings and bias the results toward the null hypothesis. Thus, the true association may be stronger than that observed in our study. A second limitation is that the serum samples were measured after being stored at −20°C for a long period. However, the Reykjavik Study confirmed the stability of CRP concentrations in serum preserved at this temperature for an average of 12 years.[27] The last limitation is that our study lacked information on drug use, which could affect serum CRP levels. It is known that several medications, including statin, angiotensin-converting enzyme inhibitors, fibrates, niacin, thiazolidinedione, and estrogen/progestogen hormone can alter CRP levels.[28] However, these medications were rarely used in our country in 1988, when the serum samples for our study were collected. This suggests that such a bias did not invalidate the present findings.

In conclusion, our study found that, in a general Japanese population, the elevation of serum hsCRP levels was an independent risk factor for future ischemic stroke in men but not for hemorrhagic stroke in either sex. The addition of elevated serum hsCRP levels to the risk factor profile may significantly increase the predictability of ischemic stroke. Moreover, our study revealed that the risk of future ischemic stroke was considerably high in subjects who had both high-hsCRP levels and another risk factor. For such individuals, an elevated serum hsCRP level may provide additional

motivation for both the treating physician and the patient to control these risk factors strictly.

Acknowledgments

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HIGH LIGHT

Antiplatelet therapy contributes to acute deterioration of intracerebral hemorrhage

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Abstract—*Objective:* The purpose of this study was to examine the effect of antiplatelet therapy on the initial severity and the acute outcome of intracerebral hemorrhage (ICH). *Methods:* The authors reviewed records of 251 consecutive patients hospitalized in their cerebrovascular center within 24 hours after onset of ICH. *Results:* Fifty-seven patients (23%) had development of ICH during oral antiplatelet therapy. The major indication for antiplatelet therapy was the prevention of stroke recurrence (63%). As compared with patients without antiplatelet therapy, those who received antiplatelet therapy more frequently were aged 70 years or older (60% vs 35%; $p < 0.001$), had previous symptomatic ischemic stroke (54% vs 7%; $p < 0.0001$), had diabetes mellitus (26% vs 15%; $p < 0.05$), and had heart disease (32% vs 8%; $p < 0.0001$). Antiplatelet therapy was predictive of an increase in the hematoma volume by more than 40% on the second hospital day (hematoma enlargement, odds ratio [OR] 7.67, 95% CI 1.62 to 36.4) and the need for emergent surgical evacuation of the hematoma (OR 3.10, 95% CI 1.18 to 8.15). Antiplatelet therapy was an independent predictor for the occurrence of any of hematoma enlargement, emergent death, or evacuation surgery, which suggests that clinical deterioration occurs into the second hospital day (OR 7.45, 95% CI 2.46 to 22.5). *Conclusions:* Antiplatelet therapy seems to contribute to the acute clinical deterioration of intracerebral hemorrhage.

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Oral anticoagulant therapy using warfarin increases the risk of intracerebral hemorrhage (ICH).[1] Patients with ICH during anticoagulant therapy have larger hematomas and worse outcomes than those not receiving anticoagulant therapy.[2,3] Similarly, meta-analyses have reported an increased risk of hemorrhagic stroke in patients using aspirin,[4,5] although another multicenter study did not confirm the increased risk of ICH with oral antiplatelet therapy.[6] The contribution of the antiplatelet therapy to the clinical severity of ICH has not yet been elucidated. Because oral antiplatelet agents are now used often in high-vascular-risk patients,[7] care should be exercised regarding their hemorrhagic complications. We sought to determine whether ICH occurring among patients taking oral antiplatelets had a worse clinical course in the acute stage vs patients not taking oral antiplatelets. We focused on the deterioration of ICH in the first 2 days, because this is the critical period when half of the 30-day mortality occurs and one-fourth of initially alert patients show a deterioration of consciousness.[8,9]

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the October 11 issue to find the title link for this article.

Methods. We reviewed records of 303 consecutive patients with nontraumatic ICH who were hospitalized in our cerebrovascular center within 24 hours after stroke onset between January 1999 and February 2005. Of the 303 patients, 52 were ineligible for the study; 21 patients were taking warfarin; 7 were receiving IV heparin, urokinase, or ozagrel (an IV thromboxane A2-synthetase inhibitor) just before the onset of ICH; 1 had development of ICH during delivery; 1 was aged 9 years; 6 hemorrhaged primarily into the ventricles; 8 had an ICH due to aneurysmal rupture; and 8 had an ICH due to vascular malformations. The remaining 251 patients (152 men and 99 women aged 66 ± 12 years) served as subjects for the current study.

In all patients, ICH was verified by CT immediately after admission to our center (CT1). CT examinations were repeated approximately 24 hours later (day 2, CT2). The number, location, and volume of the hematomas as well as the ventricular bleeding and the time interval from ICH onset to CT1 were assessed. ICH volume was determined using the ABC/2 method by neuroradiologists blinded to the clinical history.[10] An increase in the volume by more than 40% between the two CTs was defined as hematoma enlargement.[11-14] This item was not assessed for patients who died

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Table 1 *Comparison of clinical characteristics between patients with and without antiplatelet therapy*

	Antiplatelet (+), n = 57	Antiplatelet (−), n = 194	*p* Value
Baseline characteristics			
Age ≥70 y	34 (60%)	68 (35%)	<0.001
Previous symptomatic ICH	3 (5%)	26 (13%)	<0.1
Symptomatic ischemic stroke	31 (54%)	14 (7%)	<0.0001
Diabetes mellitus	15 (26%)	29 (15%)	<0.05
Heart disease	18 (32%)	16 (8%)	<0.0001
Physiologic status at admission			
Blood glucose >200 mg/dL	16 (28%)	33 (17%)	<0.07
CT findings			
Volume on CT1 >25 mL	25 (44%)	53 (27%)	<0.02
Volume on CT2 >25 mL*	6 (18%)	21 (14%)	NS
Acute outcome within 2 hospital days			
Emergent death	4 (7%)	10 (5%)	NS
Emergent evacuation surgery	20 (35%)	37 (19%)	<0.02
Hematoma enlargement >140%*	9 (27%)	12 (8%)	<0.005
Any of the above three items	33 (58%)	59 (30%)	<0.0005

* 24 patients in antiplatelet (+) and 47 patients in antiplatelet (−) were excluded.

ICH = intracerebral hemorrhage; NS = not significant.

or received surgical evacuation of the hematoma on the admission day (day 1).

Aspirin, ticlopidine, and cilostazol (a selective phosphodiesterase inhibitor) were included as target oral antiplatelets. Clopidogrel is not commercially used in Japan and was excluded from the analysis.

The following baseline characteristics were assessed: sex, age, previous symptomatic ICH, previous symptomatic ischemic stroke, hypertension (systolic blood pressure [SBP] ≥140/diastolic blood pressure [DBP] ≥90 mm Hg before ICH onset or history of antihypertensive medication), diabetes mellitus (fasting blood glucose ≥126 mg/dL, positive 75-g oral glucose tolerance test result, or history of antidiabetic medication), hypercholesterolemia (serum total cholesterol ≥220 mg/dL or history of antihypercholesterolemic medication), smoking habit, alcohol consumption, heart disease (including arrhythmia), liver disease, and neoplasm. As the physiologic status at admission, SBP, DBP, blood glucose, total cholesterol, platelets, fibrinogen, and activated partial thromboplastin time (APTT) were determined. Changes in SBP, DBP, and blood glucose during the first 2 days were also determined.

The neurologic deficits at admission were evaluated using the National Institutes of Health stroke scale (NIHSS) score. Activity of daily living before ICH was assessed by the modified Rankin scale (mRS) score.

Values are expressed as mean ± SD. Clinical characteristics of patients with ICH during oral antiplatelet treatment were compared with the remaining patients using a χ^2 test, unpaired Student's *t* test, and Mann–Whitney *U* test as appropriate. To assess initial ICH severity, we used the hematoma volume and the NIHSS score at admission. As indicators for acute deterioration of ICH, we assessed the emergent death and emergent surgical evacuation of the hematoma before the follow-up CT on day 2, and hematoma enlargement. To seek independent predictors for the above indicators, we performed multivariate logistic regression analysis using the clinical characteristics that showed a significant ($p < 0.05$) or a marginally significant ($0.05 \leq p < 0.1$) correlation with each indicator as independent variables by χ^2 test, with adjustments for sex and age. To investigate the time course of blood pressure and blood glucose, we performed one-way repeated-measures analysis of variance (ANOVA) and paired Student's *t* test for the comparison within a group, and two-way repeated-measures ANOVA for the comparison between groups.

Results. Among the 251 patients studied, 57 took oral antiplatelets daily before the onset of ICH; of these, 33 patients were taking 81 to 200 mg aspirin (81 mg in 16, 100 mg in 15, 162 mg in 1, and 200 mg in 1 patient); 12 were taking 100 to 300 mg ticlopidine (100 mg in 2, 200 mg in 9, and 300 mg in 1 patient); 3 were taking 100 mg cilostazol; 7 were taking both aspirin and ticlopidine (100 mg aspirin and 100 mg ticlopidine in 2, and 81 mg aspirin and 200 mg ticlopidine in 5 patients); 1 was taking both aspirin (100 mg) and cilostazol (100 mg); and 1 was taking both ticlopidine (200 mg) and cilostazol (200 mg). The indications for antiplatelet therapy included cardioembolic ischemic stroke in 4, noncardioembolic ischemic stroke in 32, nonvalvular atrial fibrillation without previous stroke in 4, ischemic heart disease without previous stroke in 8, and other peripheral vascular diseases in 9.

Of the baseline characteristics, age 70 years or older ($p < 0.001$), previous symptomatic ischemic stroke ($p < 0.0001$), diabetes mellitus ($p < 0.05$), and heart disease ($p < 0.0001$) were more frequent in patients taking antiplatelets than in those not taking antiplatelets (table 1; more detailed data are provided in table E-1 on the *Neurology* Web site at www.neurology.org). At admission, blood glucose greater than 200 mg/dL tended to be more frequent ($p < 0.07$), and the value of blood glucose was higher ($p < 0.02$) in patients with than without antiplatelets. In both patients with and patients without antiplatelets, SBP ($p < 0.0001$), DBP ($p < 0.0001$), and blood glucose ($p < 0.001$) became lower to the reference range during the first 2 days (figure).

The volume of the hematoma on CT1 varied from 0.3 to 252 mL (median 10.5 mL) and more frequently exceeded 25 mL in patients with than patients without antiplatelets ($p < 0.02$). The volume on CT2, varying from 0.4 to 210 mL

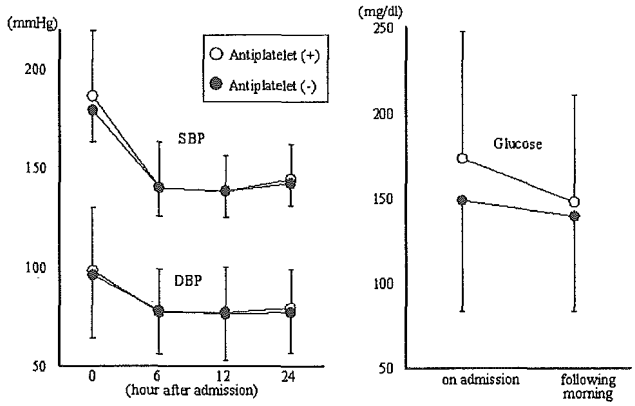

Figure. Changes in systolic (SBP) and diastolic blood pressure (DBP) and blood glucose between patients with (open circle) and without antiplatelet therapy (closed circle). Two-way repeated-measures analysis of variance (ANOVA) shows a group × time interaction for blood glucose ($p < 0.02$), not for SBP or DBP. One-way repeated-measures ANOVA shows a decrease in SBP and DBP in both groups ($p < 0.0001$). Paired t test shows a decrease in blood glucose in both groups ($p < 0.001$). Patients who died within 24 hours after admission were excluded because of the lack of 24-hour follow-up measurements.

(median 8.4 mL), was assessed for only 180 patients, because the remaining 71 died emergently or were treated by surgical evacuation. Hematoma enlargement was more common in patients who received antiplatelets ($p < 0.005$).

The NIHSS score at admission for patients receiving antiplatelets was 6 or lower in 22 patients, between 7 and 15 in 13 patients, and 16 or greater in 22 patients. For the patients not receiving antiplatelets, the NIHSS score at admission was 6 or lower in 71 patients, between 7 and 15 in 67 patients, and 16 or greater in 56 patients. Emergent surgical evacuation was more frequently needed in patients receiving antiplatelets ($p < 0.02$). Fourteen patients died within 24 hours after admission, for whom we did not perform surgical therapy because of severe thrombocytopenia due to end-stage leukemia in 1 patient with subcortical hemorrhage, severe brainstem dysfunction and coma in 6 patients with pontine hemorrhage, and refusal of surgical evacuation by the family in the remaining 7 comatose patients with putaminal or subcortical hemorrhage. For these 14 patients, we did not use hypertensive drugs or mechanical ventilation. More than half (58%) of the patients receiving antiplatelets emergently died or were treated by surgical evacuation or showed hematoma enlargement, vs 30% of patients who were not receiving antiplatelets ($p < 0.0005$). This percentage amounted to 58% (18 of 31 patients) for the patients taking 81 to 100 mg aspirin, the most prevalent antiplatelet agent and dosage in this study, and 56% (5 of 9) for the patients taking multiple antiplatelet agents.

Of the baseline clinical characteristics, physiologic status at admission, CT findings, and neurologic status, the 11 items in table E-2, A, were significantly or marginally significantly correlated with hematoma volume on CT1 greater than 25 mL using the χ^2 test. After multivariate analysis, heart disease, liver disease, glucose greater than 200 mg/dL, putaminal hemorrhage, subcortical hemorrhage, ventricular bleeding, interval between onset and CT1 less than 3 hours, and NIHSS score at admission of 16 or greater were independently related to the large hematoma. Similarly, SBP greater than 200 mmHg, pontine hemorrhage, ventricular bleeding, hematoma volume on CT1 greater than 25 mL, and mRS score before ICH of 3 or greater were independently related to NIHSS score at admission of 16 or greater (see table E-2, B). Antiplatelet therapy was not an independent predictor for these two indicators.

Using the χ^2 test, the items listed in table 2 (more detailed data are provided in table E-3) were significantly or marginally significantly correlated with the three indicators for acute deterioration of ICH. After multivariate analysis, antiplatelet therapy was independently predictive of hematoma enlargement together with platelet count less than 100,000/μL and interval between onset and CT1 less than 3 hours (see tables 2 and E-3, A). Similarly, antiplatelet therapy was independently predictive of emergent surgical evacuation together with putaminal hemorrhage and hematoma volume on CT1 greater than 25 mL (see tables 2 and E-3, B). Platelet count less than 100,000/μL and pontine hemorrhage were independently predictive of emergent death (see tables 2 and E-3, C). Multivariate analysis using 31 patients taking 81 to 100 mg aspirin and 194 patients not taking antiplatelets as subjects indicated that aspirin therapy was also independently predictive of hematoma enlargement (odds ratio [OR] 5.81, 95% CI 1.01 to 33.3) and emergent surgical evacuation (OR 5.07, 95% CI 1.48 to 17.4).

We determined the predictors for any of hematoma enlargement, emergent evacuation, or emergent death (see tables 2 and E-3, D). Antiplatelet therapy was an independent predictor for any of the above three indicators and increased 7.5-fold the risk of the occurrence of any of these three indicators. Platelet count less than 100,000/μL, putaminal hemorrhage, ventricular bleeding, hematoma volume on CT1 greater than 25 mL, interval between onset and CT1 less than 3 hours, and NIHSS score at admission of 16 or greater were other independent predictors. After multivariate analysis using 31 patients taking 81 to 100 mg aspirin and 194 patients not taking antiplatelets as subjects, aspirin therapy was also independently predictive of any of the above three indicators (OR 5.02, 95% CI 1.42 to 17.7).

Finally, we determined changes in SBP, DBP, and blood glucose among patients with and without hematoma enlargement and with emergent evacuation (figure E-1). In any groups, these variables became toward normal ranges in the follow-up measurements.

Discussion. We sought to clarify the negative effect of antiplatelet therapy on the acute clinical outcome of ICH. First, patients with ICH taking antiplatelet therapy were older and more frequently had symptomatic ischemic stroke, diabetes mellitus, and heart disease than those not taking antiplatelet therapy. Second, antiplatelet therapy was not predictive of a larger hematoma or more severe neurologic deficits at admission, both of which are indicators of the initial ICH severity. Third, antiplatelet therapy was an independent predictor for acute hematoma enlargement and emergently undergoing hematoma

Table 2 Multivariate analysis of independent predictors for acute deterioration of ICH

Item	*p* Value	OR (95% CI)
Hematoma enlargement (increase in volume >40% between two CTs)*		
Antiplatelet therapy	<0.01	7.67 (1.62–36.4)
Platelets <100,000/μL	0.024	37.8 (1.59–899.2)
ICH onset to CT1 <3 h	<0.005	8.59 (2.01–36.8)
Emergent surgical evacuation of hematoma†		
Antiplatelet therapy	0.021	3.10 (1.18–8.15)
Putaminal hemorrhage	<0.001	4.90 (1.89–12.7)
Volume on CT1 >25 mL	<0.0001	32.0 (10.7–95.4)
Emergent death‡		
Platelets <100,000/μL	0.038	19.8 (1.18–330.2)
Pontine hemorrhage	0.015	17.1 (1.74–168.4)
Any of hematoma enlargement, emergent evacuation, or emergent death§		
Antiplatelet therapy	<0.0005	7.45 (2.46–22.5)
Platelets <100,000/μL	<0.005	51.0 (3.91–666.6)
Putaminal hemorrhage	<0.0005	5.77 (2.15–15.5)
Ventricular bleeding	<0.01	3.50 (1.35–9.03)
Volume on CT1 >25 mL	<0.0001	11.0 (4.36–27.6)
ICH onset to CT1 <3 h	<0.005	3.94 (1.66–9.36)
NIHSS score at admission ≥16	0.030	2.85 (1.11–7.34)

* Symptomatic ischemic stroke, total cholesterol <130 mg/dL, fibrinogen <200 mg/dL, ventricular bleeding, volume on CT1 >25 mL, and National Institutes of Health stroke scale (NIHSS) score at admission ≥16 were significantly or marginally significantly correlated by χ^2 test but were not independently correlated after multivariate analysis.
† Liver disease, neoplasm, diastolic blood pressure >110 mm Hg, glucose >200 mg/dL, ventricular bleeding, intracerebral hemorrhage (ICH) onset to CT1 <3 hours, and NIHSS score at admission ≥16 were not independently correlated after multivariate analysis.
‡ Symptomatic ischemic stroke, systolic blood pressure >200 mm Hg, glucose >200 mg/dL, volume on CT1 >25 mL, ventricular bleeding, and NIHSS score at admission ≥16 were not independently correlated after multivariate analysis.
§ Symptomatic ischemic stroke, diabetes mellitus, liver disease, neoplasm, systolic blood pressure >200 mm Hg, and glucose >200 mg/dL were not independently correlated after multivariate analysis.

OR = odds ratio.

evacuation, as well as for the occurrence of acute hematoma enlargement, emergent death, or emergent hematoma evacuation, which indicate acute ICH deterioration within the first 2 days.

As clinical factors to affect ICH outcome, anticoagulation, hematoma size, patient age, level of consciousness, hypertension, diabetes, admission to a neurologic intensive care unit, and location within the brainstem were reported.[3] Clinical and biologic markers of the inflammatory reaction have recently been reported to be predictive of early neurologic deterioration in ICH patients.[15] However, antiplatelets have not up to now been shown to affect ICH outcome. One reason why antiplatelet-related ICH has been understudied may be the low percentage of patients with ICH receiving antiplatelet therapy. For example, a study performed within the past decade reported that less than 5% of patients with ICH were taking antiplatelets.[12] A recent study reported that 30% of the patients with ICH were taking antiplatelets.[3] During the past few years, the use of antiplatelets has become prevalent because oral antiplatelets have proven protective in high-vascular-risk patients, including those with acute and chronic stroke.[16,17] Consequently, the association of antiplatelet therapy with the initial severity and clinical outcome of ICH has become a central issue.

Although active bleeding of ICH is generally thought to cease within the first few hours, several studies reported that between 14% and 38% of patients show hematoma enlargement on the second-day follow-up CT.[12-14,18,19] Long-term antiplatelet therapy suppresses platelet function and seems to enhance active bleeding in the hyperacute stage occurring after the initial CT is performed. In the current study, the frequency of hematoma enlargement was not high even for patients receiving antiplatelet therapy (24%). An explanation for the low frequency of hematoma enlargement may be the exclusion from the analysis of many patients with potential hematoma enlargement because of emergent death or surgery. A severe mass effect often causes emergent death and surgery, and the mass effect seems to be associated with a large hematoma at admission, hematoma enlargement after admission, and secondary edema formation. An acute mass effect occurring

within 2 days was reported to be associated with hematoma enlargement, whereas a later mass effect was reported to be associated with an increase in edema.[20]

In table 2 (table E-3, D), we identify the seven independent predictors for acute ICH deterioration. Among them, antiplatelet therapy and a low platelet level might alter platelet function and consequently prolong active bleeding. Putaminal hemorrhage and large hematoma at admission might reflect a severe mass effect and consequent emergent death or surgery. The relationship between hematoma enlargement and initial hematoma size has been disputed.[12,14,18] Ventricular bleeding might cause hydrocephalus and worsen patient outcome. An early visit after onset seems to increase the opportunity for continuous active bleeding after the initial CT. In addition, patients with severe initial symptoms seem to visit hospital emergency departments often, although an early visit after onset was not predictive of severe neurologic deficits in this study. In the current analysis, various comorbidities including previous cerebrovascular and cardiovascular disease and risk factors for arteriosclerosis were not independent predictors for acute ICH deterioration except for thrombocytopenia.

Insufficient control of physiologic variables in the hyperacute stage, as well as their initial severity, could have affected the risk of hematoma expansion and clinical deterioration. We principally decreased SBP to below 150 mm Hg in acute ICH patients, which seems to be optimal in preventing hematoma growth,[14] and similarly decreased blood glucose toward the reference range (see figures 1 and E-1). Thus, differential control of these variables did not seem to essentially cause difference in the acute outcome in our patients.

The results of the current study warn about the risk of ICH deterioration in patients receiving antiplatelet therapy and suggest that the next logical step would be to determine the relative importance in relation to ICH deterioration of antiplatelet agent dosage and the use of multiple antiplatelets. These issues were not fully examined in this study because of the small number of patients enrolled. In addition, a few patients took an excess dosage of antiplatelets compared with the recommended dosage in Japan (e.g., 300 mg ticlopidine), and it might affect generalization of the current results. The current results showed that 81 to 100 mg aspirin, the most prevalent antiplatelet agent and dosage in this study, was independently predictive of acute ICH deterioration within the first 2 days. Prospective trials using large populations with antiplatelets are needed to overcome these limitations and clarify unresolved issues in this study, including outcome of ICH according to antiplatelet agent and dosage.

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PAPER

Ten year recurrence after first ever stroke in a Japanese community: the Hisayama study

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Background: Very few population based cohort studies have focused on the long term recurrence of stroke.
Objective: To examine 10 year cumulative recurrence rates for stroke in a Japanese cohort according to pathological type and clinical subtype of brain infarction.
Methods: During a 32 year follow up of 1621 subjects ≥40 years of age, 410 developed first ever stroke. These were followed up prospectively for 10 years after stroke onset.
Results: During follow up, 108 (26%) experienced recurrent stroke. The cumulative recurrence rates were 35.3% at five years and 51.3% at 10 years. The 10 year recurrence rates of subarachnoid haemorrhage (SAH), brain haemorrhage, and brain infarction were 70.0%, 55.6%, and 49.7%, respectively; the difference between SAH and brain infarction was significant ($p=0.004$). Most recurrent episodes after SAH or brain haemorrhage happened within a year after the index stroke, whereas recurrence of brain infarction increased consistently throughout the observation period. Cardioembolic stroke had a higher recurrence rate (75.2%) than lacunar infarction (46.8%) ($p=0.049$). The 10 year risk of stroke recurrence increased with age after lacunar or atherothrombotic brain infarction, but not after the other types or subtypes. After atherothrombotic brain infarction, cardioembolic stroke, or SAH, the type and subtype of most recurrent strokes were the same as for the index stroke, but recurrence after lacunar infarction or brain haemorrhage showed divergent patterns.
Conclusions: Japanese people have higher recurrence rates of stroke than other populations. Recurrence rate after a first brain infarct increases consistently through the next 10 years.

Japanese people have high rates of morbidity and mortality from stroke.[1] Among stroke survivors, recurrence is common, resulting in cumulative disability and cognitive dysfunction.[2] Consequently, precise information is needed on the long term rates and determinants of recurrence after first stroke, so that clinical trials can be designed and health care policies for primary and secondary stroke prevention can be established. Most studies on stroke recurrence, reported mainly from Western countries, have been based on stroke registries[3–11] or on series of patients referred to hospitals.[12 13] A truly representative assessment of stroke recurrence in a community would require a prospective cohort of a defined population and an exhaustive follow up system. The Framingham study is the only cohort based examination of both initial and recurrent stroke, but it refers to the recurrence of thrombotic brain infarction only.[14] Stroke is divided into several pathological types. Among them, brain infarction is further classified into several clinical subtypes.[15–17] Very few studies, however, have accurately defined types and subtypes while also evaluating the long term risk of stroke recurrence.[3]

Since 1961, we have been carrying out a prospective cohort study of cardiovascular disease in the town of Hisayama, Japan.[18 19] The most outstanding features of this study are that the causes of death were verified by necropsy and that most of the stroke patients were examined morphologically at necropsy or, before death, by brain imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). Our aim in this study was to estimate 10 year cumulative recurrence rates after first ever stroke in the community of Hisayama, using data stratified by sex, age, stroke type, and, in cases of brain infarction, the clinical subtype.

METHODS

Subjects and follow up surveys

In 1961, we carried out a screening examination among Hisayama residents and established a cohort consisting of 1621 stroke-free subjects aged ≥40 years (88.1% of the total population in this age range). These subjects were then followed up for 32 years, from 1 November 1961 to 31 October 1993. A detailed description of the study methods has been published previously.[18 19] In brief, we collected information about new cardiovascular events through a daily monitoring system established by the study team, local practitioners, and the town government. When we suspected a patient was having a new neurological symptom or a new deterioration of an already existing symptom, one of the physicians participating in the study would carefully evaluate the subject and try to obtain information by further diagnostic examinations, including lumbar puncture, cerebral angiography, or recent brain CT or MRI. During the 32 year period, all but two subjects were followed up and 1063 subjects died. Of those who died, 861 (81.0%) underwent necropsy.

The study was conducted with the approval of the human ethics review committee of Kyushu University Graduate School of Medical Sciences.

First ever stroke

Stroke, defined as the sudden onset of a non-convulsive and focal neurological deficit persisting for over 24 hours, was classified into four pathological types: brain infarction, brain haemorrhage, subarachnoid haemorrhage, and undetermined. Brain infarction was further divided into four clinical subtypes: lacunar infarction, atherothrombotic brain

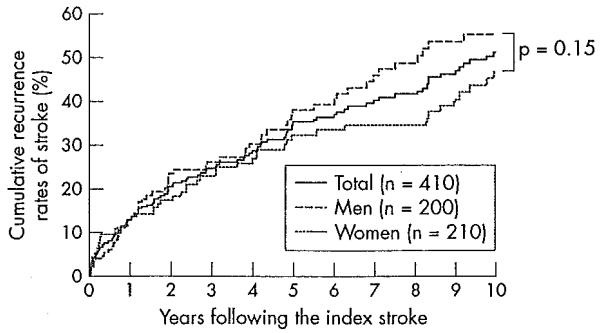

Figure 1 Kaplan–Meier estimates of cumulative recurrence rates of stroke for all subjects and for all subjects divided by sex. Deaths without stroke recurrence were censored.

infarction, cardioembolic stroke, and undetermined. These types and subtypes were defined on the basis of the *Classification of Cerebrovascular Disease III* proposed by the National Institute of Neurological Disorders and Stroke (USA).[15] The subtypes of ischaemic stroke were classified by TOAST (trial of Org 10172 in acute stroke treatment)[16] and by the Cerebral Embolism Task Force.[17] A detailed method of classifying stroke has been published previously.[19] The diagnosis and classification of stroke in our study were based on clinical history, neurological examination, all available clinical information (including brain CT or MRI), and necropsy findings.

During the 32 year follow up, we identified 410 first ever stroke events (200 men and 210 women, mean (SD) age, 73.9 (10.1) years), and divided them into 298 cases of brain infarction, 73 of brain haemorrhage, 35 of subarachnoid haemorrhage, and four undetermined. The cases of brain infarction by subtype consisted of 167 lacunar infarcts, 62 atherothrombotic brain infarcts, 56 cardioembolic strokes, and 13 undetermined.

Recurrent stroke

The definition of recurrent stroke was the same as that of index stroke, but with an additional criterion: there had to be either a new focal neurological deficit or a new deterioration of a previous deficit that was not attributed to brain oedema, haemorrhagic transformation after ischaemia, intercurrent illness, or iatrogenesis. This definition included recurrence in the early stage after the preceding stroke or recurrence in the same vascular territory as the preceding stroke.

We followed up the 410 patients with index stroke from the time of stroke onset until death or 31 August 2003. Under those conditions, all patients completed the follow up period. In the 10 years after the index stroke, 108 patients developed recurrent stroke. Of these, 88 had one recurrent stroke, 13 had two, six had three, and one had four. However, the end point of this study for each subject was the first recurrence.

Morphological evaluation

Brain imaging, including CT or MRI, was carried out in 153 (37%) of the 410 subjects with index stroke and in 43 (40%) of the 108 subjects with recurrent stroke. Necropsy findings were available in 332 (84%) of the 394 deceased stroke patients. As a result, morphological evaluation, including brain imaging or necropsy, was undertaken in 376 (92%) of the index stroke patients and 102 (94%) of the recurrent stroke patients until 31 August 2003.

Because we began collecting data on stroke subjects in 1961, imaging examinations of the brain and heart were non-existent in the early study period. However, we compensated

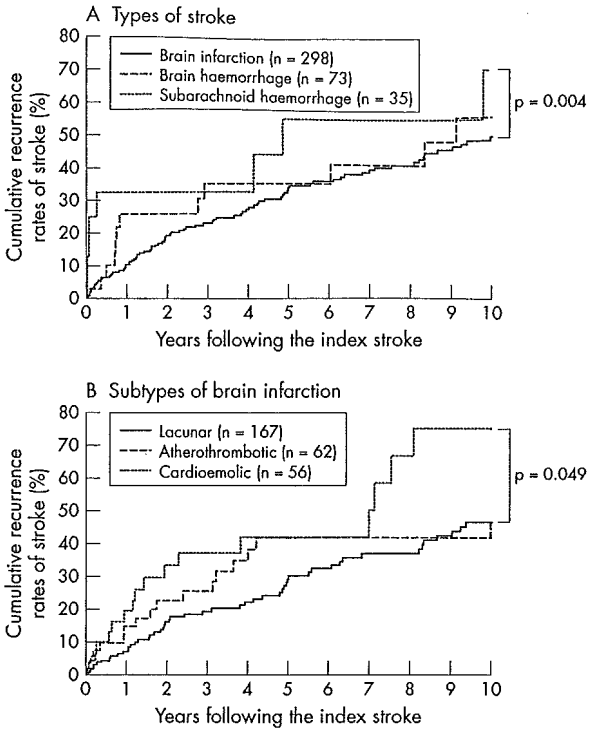

Figure 2 Kaplan–Meier estimates of cumulative recurrence rates of stroke according to stroke type (A) and, in cases of brain infarction, the subtype (B). Deaths without stroke recurrence were censored.

for this disadvantage by carrying out necropsy examinations on the vast majority of deceased patients. We reviewed the brains to evaluate the site, size, and pathological features of the stroke. We also investigated the heart and major vessels in detail—including the aorta, carotids, vertebrobasilar arteries, and the circle of Willis—in order to identify atherothrombotic stenotic lesions and embolic sources. In cases where the necropsy was carried out a long time after stroke onset, it was important to distinguish brain haemorrhage from brain infarction with haemorrhagic transformation. The latter was usually the result of a cardioembolic mechanism. When an infarcted area was surrounded by deposition of haemosiderin—with either no or mild atherosclerosis of the responsible artery, and given the presence of the embolic source—we considered the stroke lesion to be a brain infarct with haemorrhagic transformation. An old lesion that looked like a slit was considered to indicate a brain haemorrhage, especially if found in the basal ganglia or thalamus.

To classify the subtypes of brain infarction, we considered important the size and location of the infarcted area, the presence of stenosis or occlusion of a responsible cerebral artery, and the embolic source, in addition to clinical information including the disease course. Where multiple asymptomatic infarctions were present, we considered an infarct to be the lesion responsible for the stroke when it was most closely in accord with the neurological findings and disease course in the acute period of the stroke. The criteria for diagnosing brain infarction subtypes were given in full detail in our previous report.[19] When sufficient clinical and morphological information was obtained, a diagnosis of subtype was defined as "definite"; on the other hand, when either type of information was insufficient, the diagnostic level was defined as "probable." Among 298 cases of brain infarction, 272 were definite and 26 probable. In this study,

we present the data on the definite and probable cases together, as these combined data were almost identical to the data for definite cases only.

Statistical analysis

SAS software (version 6.12) was used for statistical analysis. The cumulative recurrence rates of stroke and the 95% confidence intervals (CI) were estimated by the Kaplan–Meier product limit method. The Cox proportional hazards model was used to test differences in recurrence rates as well as to estimate relative risks (RR) and 95% CIs of stroke recurrence.

RESULTS

Recurrence rates of stroke

Figure 1 shows the Kaplan–Meier estimates of cumulative recurrence rates of stroke for all subjects and for all subjects divided by sex. The recurrence rates (95% CI) at 1, 5, and 10 years were 12.8% (8.9% to 16.6%), 35.3% (29.0% to 41.5%), and 51.3% (43.8% to 58.9%), respectively, for all subjects. For men, these rates were 12.9% (7.3% to 18.5%), 38.1% (28.9% to 47.2%), and 55.6% (44.9% to 66.4%); for women the rates were 12.5% (7.3% to 17.6%), 32.3% (23.8% to 40.9%), and 47.1% (36.5% to 57.6%). The recurrence rates were slightly higher for men than for women, but the overall difference was not statistically significant ($p = 0.15$).

Figure 2, panel A, shows cumulative recurrence rates of stroke by type of index stroke. The recurrence rates at 1, 5, and 10 years were 10.0% (6.3% to 13.8%), 34.1% (27.3% to 40.9%), and 49.7% (41.4% to 57.9%) after brain infarction; 25.6% (9.0% to 42.2%), 34.9% (16.0% to 53.8%), and 55.6% (32.2% to 79.1%) after brain haemorrhage; and 32.5% (10.3% to 54.6%), 55.0% (25.6% to 84.4%), and 70.0% (39.0% to 100%) after subarachnoid haemorrhage, respectively. The 10 year recurrence rate of subarachnoid haemorrhage was significantly higher than that of brain infarction (RR = 2.89 (95% CI, 1.40 to 5.97); $p = 0.004$). Also, brain haemorrhage recurred at a slightly higher rate than brain infarction, but the difference was not statistically significant ($p = 0.52$). Annual recurrence rates after brain infarction were about 10% per year in the first two years and consistently about 4% per year afterward. On the other hand, 58.3% of recurrent episodes took place within a year after brain haemorrhage, and 66.7% within three months after subarachnoid haemorrhage.

Figure 2, panel B, shows the cumulative recurrence rates of stroke by clinical subtype of brain infarction. The recurrence rates at 1, 5, and 10 years were 7.2% (3.1% to 11.2%), 30.4% (22.1% to 38.7%), and 46.8% (36.6% to 56.9%) after lacunar infarction; 14.8% (4.5% to 25.0%), 42.0% (25.5% to 58.5%), and 46.9% (29.2% to 64.5%) after atherothrombotic brain infarction; and 19.6% (6.3% to 32.8%), 42.2% (23.8% to 60.6%), and 75.2% (52.6% to 97.8%) after cardioembolic stroke, respectively. Cardioembolic stroke had a significantly higher risk of 10 year recurrence than lacunar infarction (RR = 1.76 (95% CI, 1.00 to 3.11); $p = 0.049$). The recurrence rate of atherothrombotic brain infarction was slightly higher than that of lacunar infarction, but the difference was not statistically significant ($p = 0.59$).

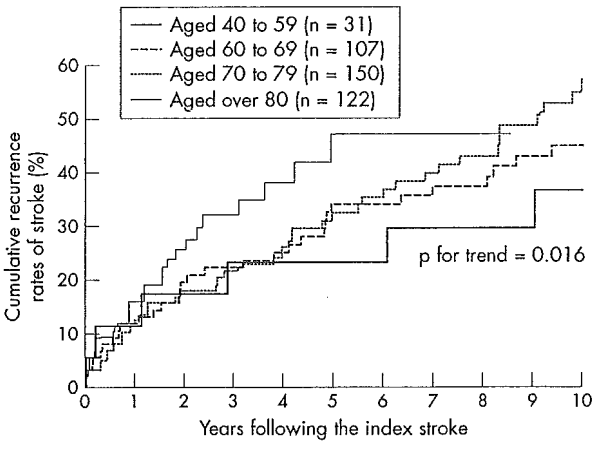

Figure 3 Kaplan–Meier estimates of cumulative recurrence rates of stroke for all subjects divided by age. Deaths without stroke recurrence were censored.

Figure 3 shows the cumulative recurrence rates of stroke by age. The 10 year risk of stroke recurrence was lowest in the youngest age group (40 to 59 years) and increased with age. Table 1 shows the relative risks of stroke recurrence among age groups during 10 years for each type and subtype of index stroke. The 10 year risk of stroke recurrence after brain infarction was lowest in the youngest age group and increased with age. For brain haemorrhage or subarachnoid haemorrhage, on the other hand, there was no significant relation between age and recurrence rates. Among the subtypes of brain infarction, the 10 year risk of recurrence after lacunar and atherothrombotic brain infarction was lowest in the youngest age group and increased with age, whereas for cardioembolic stroke there was no significant relation between age and recurrence rates.

Patterns of stroke recurrence

To evaluate patterns of stroke recurrence, table 2 shows the numbers and frequencies of first recurrent stroke by pathological types and clinical subtypes according to the type of index stroke. Most recurrent strokes after atherothrombotic brain infarction, cardioembolic stroke, or subarachnoid haemorrhage were the same type or subtype as the index stroke. On the other hand, recurrence after lacunar infarction or brain haemorrhage showed divergent patterns. The 51 patients who had recurrent stroke after lacunar infarction were divided as follows: 18 cases (35%) had a second lacunar infarction, 16 (31%) had atherothrombotic brain infarction, nine (18%) had brain haemorrhage, and six (12%) had cardioembolic stroke. Among the 12 recurrent cases of brain haemorrhage, seven (58%) had a second brain haemorrhage, three (25%) had lacunar infarction, and two (17%) had atherothrombotic or cardioembolic infarction.

DISCUSSION

One of the strengths of our study is that we investigated almost all stroke events occurring in a community based prospective cohort. Our study design eliminated the selection bias encountered in stroke registries or in series of hospital inpatients. Another strength is that recurrence rates were estimated up to 10 years after a subject's first ever stroke.

Recurrence rates of stroke

Three previous reports from stroke registries in Australia[3] and Britain[4 5] have reported five year cumulative stroke recurrence rates of 16.6% to 29.5%. In comparison, our study's five year cumulative stroke recurrence rate was 35.3%. There might be several reasons for this difference. First, there was a difference in methodology. The studies of the other three stroke registries all used a single set of criteria, which excluded vascular events occurring in the first 21 days after the index stroke unless such an event was clearly in a different vascular territory.[3–5] On the other hand, our study excluded neither early recurrence (10 cases within 21 days) nor recurrence in the same vascular territory. Second, race might greatly influence stroke recurrence. In our study,

Table 1 Relative risks and 95% confidence intervals of stroke recurrence during 10 years by age in each type or subtype of index stroke

	Age group (years)				
	40 to 59	60 to 69	70 to 79	80 and over	p Value
Index stroke	RR	RR (95% CI)	RR (95% CI)	RR (95% CI)	for trend
All types of stroke	1.0	1.3 (0.5 to 3.0)	1.6 (0.7 to 3.8)	2.2 (0.9 to 5.4)	0.016
Brain infarction	1.0	2.0 (0.6 to 6.5)	2.5 (0.7 to 8.1)	3.9 (1.1 to 13.1)	0.002
Lacunar infarction	1.0	2.2 (0.5 to 9.4)	2.6 (0.6 to 11.1)	4.8 (1.0 to 22.2)	0.022
Atherothrombotic brain infarction	1.0 *		1.8 (0.4 to 7.5)	4.7 (1.2 to 18.6)	0.001
Cardioembolic stroke	1.0	0.8 (0.1 to 7.3)	1.4 (0.2 to 12.3)	0.4 (0.0 to 4.1)	0.51
Brain haemorrhage	1.0	0.6 (0.0 to 6.3)	1.2 (0.2 to 10.3)	2.1 (0.2 to 24.3)	0.71
Subarachnoid haemorrhage	1.0	1.0 (0.2 to 6.0)	0.7 (0.1 to 4.4)	0.0	0.60

*Two age groups (40 to 59 and 60 to 69) were combined, as there were no recurrences after atherothrombotic brain infarction in the 40 to 59 age group.
CI, confidence interval; RR, relative risk.

haemorrhagic stroke—including brain haemorrhage and subarachnoid haemorrhage—recurred at higher rates than brain infarction, and the proportion of haemorrhagic stroke (26%) among all types was higher than those found in the three registries in Western countries (14% to 19%).[3–5] In addition, as Asians, including Japanese, have a higher stroke incidence than Europids,[1] they might also have higher rates of stroke recurrence.

In our study, most recurrent episodes occurred within a year after the index haemorrhagic stroke. This may indicate the importance of controlling risk factors and of treating the patient to prevent recurrence without delay in the first days and months after the onset of haemorrhagic stroke. On the other hand, cumulative recurrence rates after brain infarction, especially lacunar infarction, increased steadily during our 10 year study period. The Oxfordshire Community Stroke Project[6] also showed that the recurrence rate after lacunar infarction was low and almost constant throughout the follow up period. Arteriosclerosis, which is thought to progress consistently for a long period, may be related to recurrent thrombotic infarction. Thus careful observation and adequate treatment to prevent recurrence are needed for a long time after brain infarction.

Several studies have focused on the relations between brain infarction subtypes and the risks of recurrent stroke,[3 7–10 12] but their findings are equivocal. Some of those studies have claimed that the subtype of brain infarction is not a predictor of long term recurrence,[3 7 8] while others showed that the highest risk of recurrence is with atherothrombotic brain infarction.[9 10 12] In our study, cardioembolic stroke had the highest risk of recurrence among the three major subtypes of brain infarction. This is probably attributable to our inclusion of early recurrent episodes, which were often observed after cardioembolic stroke.[20 21]

In some studies,[3 11] aging was found to be a predictor of stroke recurrence. In the present study, the risk of recurrence after first ever lacunar or atherothrombotic brain infarction was lowest in the youngest age group and then increased with age. Aging would accelerate atherosclerotic changes in major cerebral arteries and arteriolosclerotic changes in penetrating arteries, thus increasing the risk of recurrent stroke.

Patterns of stroke recurrence

In the present study, the types or subtypes of most recurrent strokes after atherothrombotic brain infarction, cardioembolic stroke, or subarachnoid haemorrhage were the same as those of the index stroke. On the other hand, recurrence after lacunar infarction or brain haemorrhage showed divergent patterns. This finding was also emphasised in some previous reports.[4 13]

Several aetiological mechanisms for lacunar infarction have been proposed[22–24]: lipohyalinosis or microatheroma in a penetrating artery; branch-atheromatous disease, which is located in basilar or middle cerebral arteries and occludes the origins of one or more penetrating arteries; and microembolism from carotid or cardiac disease. These multifactorial aetiologies would support divergence in the type and subtype of recurrent stroke after lacunar infarction. Our findings denote the importance of evaluation to detect any large vessel disease or embolic source, even in patients with lacunar infarction.

Table 2 The numbers and frequencies of first recurrent stroke by pathological types and clinical subtypes according to type of index stroke

	Type or subtype of recurrent stroke								
		Subtype of BI							
Type or subtype of index stroke	All BI	LA	AT	CE	UND-BI	BH	SAH	UND	Total
Brain infarction	74 (85%)					10 (11%)	–	3 (3%)	87 (100%)
Lacunar infarction		18 (35%)	16 (31%)	6 (12%)	–	9 (18%)	–	2 (4%)	51 (100%)
Atherothrombotic brain infarction		1 (6%)	14 (82%)	–	1 (6%)	1 (6%)	–	–	17 (100%)
Cardioembolic stroke		–	–	16 (94%)	1 (6%)	–	–	–	17 (100%)
Undetermined subtype of BI (UND-BI)		–	–	–	1 (50%)	–	–	1 (50%)	2 (100%)
Brain haemorrhage	5	3 (25%)	1 (8%)	1 (8%)	–	7 (58%)	–	–	12 (100%)
Subarachnoid haemorrhage	2	1 (11%)	1 (11%)	–	–	1 (11%)	6 (67%)	–	9 (100%)
Undetermined type of stroke	–	–	–	–	–	–	–	–	0 (0%)

Percentages are the proportions of types or subtypes of recurrent stroke calculated using the numbers of total recurrent stroke as the denominators.
AT, atherothrombotic brain infarction; BH, brain haemorrhage; BI, brain infarction; CE, cardioembolic stroke; LA, lacunar infarction; SAH, subarachnoid haemorrhage; UND, undetermined.

Hypertension is a major risk factor for both lacunar infarction and brain haemorrhage, and lesions of all lacunar infarcts and most brain haemorrhages in our patients were located in brain areas that have the common feature of penetrating arteries, such as the basal ganglia, thalamus, and pons. These similarities would support the overlap between lacunar infarction and brain haemorrhage in recurrent stroke types.

Study limitations

There are several potential limitations to the findings in our study. First, we enrolled stroke cases that developed among an inception cohort during 32 years of follow up. The prevalence of cardiovascular risk factors and the risk of stroke recurrence may have changed widely during this long term observation period.[25] Secular trends in stroke recurrence should be examined, and we will do so in another study. Second, the study did not consider the effects of cardiovascular risk factors or those of medical or surgical treatment. Thus our estimates for the risk of stroke recurrence are probably quite conservative. Third, brain imaging was available in only 37% of the index stroke cases. However, we collected available clinical information on both index and recurrent strokes in minute detail and carried out necropsies on 84% of deceased stroke patients. We believe that our exhaustive and careful evaluation of the clinical information, as well as the high rate of necropsy, improved the quality and validity of the diagnosis as well as the stroke classification in our study.

Conclusions

Our findings show higher recurrence rates of stroke in a Japanese community than in Western populations. The divergent patterns of stroke recurrence after index lacunar infarction or brain haemorrhage are of interest and importance for the prevention of recurrent stroke, because the Japanese are characterised by high morbidity of lacunar infarction and brain haemorrhage. The consistent increase in cumulative recurrence rates during the long observation period and the higher recurrence rates after index brain infarction among older patients are both important for medical care. We believe that these findings will contribute to a better understanding of stroke recurrence in the Japanese, who are considered to be at greater risk of stroke than other populations.

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