Original Article

Small Dense Low-Density Lipoproteins Cholesterol can Predict Incident Cardiovascular Disease in an Urban Japanese Cohort: The Suita Study

Hidenori Arai[1], Yoshihiro Kokubo[2], Makoto Watanabe[2], Tatsuya Sawamura[3], Yasuki Ito[4], Asako Minagawa[4], Tomonori Okamura[5] and Yoshihiro Miyamato[2]

[1]Department of Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan
[2]Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Suita, Japan
[3]Department of Vascular Physiology, National Cerebral and Cardiovascular Center, Suita, Japan
[4]Research and Development Department, Denka Seiken, Tokyo, Japan
[5]Department of Preventive Medicine and Public Health, School of Medicine, Keio University, Tokyo, Japan

Aim: Several lines of evidence indicate that small dense low-density lipoproteins (sd-LDL) are more atherogenic than large buoyant LDL; however, few prospective studies have addressed the role of sd-LDL in cardiovascular disease (CVD). We therefore examined the association between sd-LDL cholesterol (sd-LDL-C) and CVD in a Japanese cohort.

Methods: An 11.7-year prospective study was performed using a general population aged 30-79 without a history of cardiovascular disease. Direct LDL-C and sd-LDL-C were measured in samples from 2034 participants (968 men and 1066 women).

Results: During the follow-up period, there were 116 incident cases of CVD. The multivariable-adjusted hazard ratios (HRs) of sd-LDL-C for CVD were calculated using a proportional hazards regression model after adjusting for age, hypertension, diabetes, use of lipid-lowering drugs, body mass index, and current smoking and alcohol drinking, and found that increasing quartiles of sd-LDL-C were associated with increased risk of CVD. We also determined that age and sex-adjusted HRs per 10 mg/dL of sd-LDL-C and HRs for CVD, stroke, cerebral infarction, and coronary artery disease were 1.21 (95% CI: 1.12-1.31), 1.17 (95% CI: 1.05-1.30), 1.15 (95% CI: 1.00-1.33), and 1.29 (95% CI: 1.14-1.45), respectively.

Conclusions: It was demonstrated that sd-LDL-C was significantly associated with CVD in a Japanese population, providing evidence of sd-LDL-C as an important biomarker to predict CVD.

J Atheroscler Thromb, 2013; 20:195-203.

Key words; Cardiovascular disease, Lipoproteins, Lipids, Risk factors, Epidemiology

Introduction

The causal relationship between high levels of serum low-density lipoprotein cholesterol (LDL-C) and cardiovascular disease (CVD) has been well established in previous cohort studies[1-5]. Recent clinical trials have also indicated significant event reduction by statins in the primary and secondary prevention of CVD[6-8]; therefore, LDL-C is one of the most important risk factors of CVD and many guidelines, including ours, recommend certain target LDL-C goals for risk management to prevent the development of CVD[5].

Although we use LDL-C as the primary target for cholesterol-lowering therapy, LDL particles are heterogeneous with respect to size and density. Compared to large, buoyant LDL, small dense LDL (sd-LDL) particles exhibit a prolonged plasma residence time, increased penetration into the arterial wall, lower affinity for the LDL receptor, and increased sus-

Address for correspondence: Hidenori Arai, Department of Human Health Sciences, Kyoto University Graduate School of Medicine, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
E-mail: harai@kuhp.kyoto-u.ac.jp
Received: June 20, 2012
Accepted for publication: September 6, 2012

ceptibility to oxidation[9]. Thus, sd-LDL particles possess elevated atherogenic potential. Furthermore, elevated concentrations of sd-LDL can be found in patients with type 2 diabetes, metabolic syndrome, chronic kidney disease, and familial combined hyperlipidemia[10-14], all of which have been found as highly atherogenic conditions. Although Hirano *et al.* showed that sd-LDL-C is significantly higher in patients with coronary artery disease (CAD) in a cross-sectional study[14], no prospective study has addressed whether sd-LDL-C can predict a risk for CVD in non-Western populations. Recently, the Québec Cardiovascular Study has shown prospectively that men with an elevated proportion of LDL with a diameter less than 25.5 nm had a 3.6-fold increased risk of CAD compared with men with relatively normal LDL[15], indicating the strong link of sd-LDL to CVD as a biomarker of cardiovascular disease. Due to its atherogenic properties it is useful to measure sd-LDL for risk assessment; however, a reliable routine method is lacking.

sd-LDL has been measured by ultracentrifugation[16] or gradient gel electrophoresis[17]; however, these methods are both unsuitable for routine analysis, because each requires expensive equipment, complicated techniques, and long assay times. Hirano *et al.* have recently developed a simple precipitation method for sd-LDL-C quantification consisting of 2 steps: removal of apolipoprotein B-containing sd-LDL-free lipoproteins by precipitation with heparin and magnesium, followed by LDL-C measurement by the homogeneous method[18, 19]. This assay allowed us to screen sd-LDL-C in a large cohort. Using this assay, Ai *et al.* recently performed a case control study using samples from the Framingham Offspring Study and found significantly higher sd-LDL-C in women with CAD[20]. Koba *et al.* also showed that sd-LDL-C is more powerful than LDL-C for the determination of CAD[21]; however, these are cross-sectional studies and a prospective study is required to determine whether sd-LDL is an independent predictor of CVD. Therefore, the aim of this study was to address the role of sd-LDL-C for incident CVD in a large cohort study in Japan, the Suita study.

Methods

Population

The Suita study, a cohort study on CVD of urban residents, was established in 1989. The details of this study have been described elsewhere[22]. Briefly, 6485 men and women aged 30-79 years underwent a baseline survey at the National Cerebral and Cardiovascular Center between September 1989 and March 1994, and received medical examinations every 2 years. For these participants, we set the baseline of the present study at medical examinations held between April 1994 and February 1995, since at that time serum samples were collected and stored at −80℃. During this period, 2,437 participants attended the medical examination and were followed until the end of 2007. Of these, 403 participants were excluded due to the following reasons: history of CAD or stroke (n=106), lost to follow-up (n=132), and other reasons such as missing data (n=165). Data from the remaining 2,034 participants (968 men and 1,066 women) were included in the analysis. Informed consent was obtained from all participants. This cohort study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

Baseline Examination

Blood samples were collected after the participants had fasted for at least 10 hours. The samples were centrifuged immediately. Blood pressure was measured in triplicate on the right arm after 5 min of rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used for analysis. At baseline examination, subjects were classified into one of the 5 blood pressure categories based on the criteria of ESH-ESC 2007: optimal (SBP < 120 mmHg and DBP < 80 mmHg), normal (SBP 120-129 mmHg or DBP 80-84 mmHg), high-normal blood pressure (SBP 130-139 mmHg or DBP 85-89 mmHg), hypertension grade 1 (SBP 140-159 mmHg or DBP 90-99 mmHg), or hypertension grade ≥2 (SBP ≥160 mmHg or DBP ≥100 mmHg). Antihypertensive drug users were classified according to their blood pressure at the baseline survey. Diabetes was defined as fasting serum glucose ≥7.0 mmol/L (126 mg/dL) or current use of medications for diabetes. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Well-trained health nurses obtained information on smoking, drinking, and medical histories.

Laboratory Measurements

Serum total cholesterol, triglyceride, and HDL cholesterol (HDL-C) were determined by standard enzymatic methods. Serum glucose was also measured. For the purposes of this study, we used archived plasma samples that had been frozen at −80℃ and never previously thawed for the assessment of direct LDL-C and sd-LDL-C by homogeneous methods on a Hitachi 7180 automated analyzer (Hitachi, Tokyo, Japan)[18, 19]. The kits used for these tests (LDL-C and sd-LDL-C) were provided by Denka Seiken (Tokyo, Japan). Assays

for direct LDL-C and sd-LDL-C were previously calibrated and directly compared with concentrations obtained after isolation of LDL and sd-LDL by ultracentrifugation.

Endpoint Determination

As previously reported, the endpoints of the present study were (1) date of first CAD or stroke event; (2) date of death; (3) date of leaving Suita city; and (4) the end of December 2007. The first step in the survey for CAD and stroke involved checking the health status of all participants by repeated clinical visits every two years and yearly questionnaires by mail or telephone. In the second step, in-hospital medical records of participants who were suspected of having CAD were reviewed by registered hospital physicians or research physicians who were blinded to the baseline information. The criteria for a diagnosis of CAD included first-ever acute myocardial infarction, sudden cardiac death within 24 h after the onset of acute illness, or coronary artery disease followed by coronary artery bypass surgery or angioplasty. The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project[23]. The criteria for stroke were defined according to the US National Survey of Stroke criteria[24]. Classification of patients into stroke subtypes was based on examination of computed tomography, magnetic resonance imaging, or autopsy.

Statistical Analysis

Continuous variables between groups were compared by analysis of variance and categorical variables were compared by a chi-square test. Triglyceride levels were logarithmically transformed to improve the skewed distribution. The hazard ratio (HR) for MI or stroke was calculated using a proportional hazards model adjusted for age, sex, hypertension (dichotomous variable), diabetes, HDL-C, BMI, smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker). All confidence intervals were estimated at the 95% level and significance was set at $p<0.05$. All statistical analyses were conducted using the SAS statistical software package (release version 8.2; SAS Institute, Cary, NC, USA).

Results

Baseline Clinical Characteristics According to sd-LDL-C Quartiles

To study the role of sd-LDL in the incidence of CVD, we divided the cohort into quartiles according to the basal level of sd-LDL-C. **Table 1** shows the clinical characteristics and cardiovascular risk factors of the study population according to the quartiles of sd-LDL-C. BMI, total cholesterol, LDL-C, and triglyceride significantly increased across the sd-LDL-C quartiles both in men and women, while HDL-C decreased in both genders. A significant trend was observed across the quartiles for the severity of high blood pressure, lipid-lowering drug use, and prevalence of diabetes at baseline both in men and women; however, a significant trend for age was only found in women, not in men.

Incidence of CVD According to sd-LDL-C Quartiles

To confirm our previous study, the association between LDL-C and CAD was examined by dividing the cohort into quartiles according to the baseline LDL-C. It was found that age- and multivariable-adjusted HRs for CAD were statistically significant only in men, not in women or the total cohort. The HR of the 4th quartile in men was 3.53 (95% confidence intervals (CIs): 1.31-9.54) in an age-adjusted model and 3.56 (95% CIs: 1.28-9.86) in a multivariable-adjusted model, consistent with our previous report[1]. We then performed analysis to examine the effect of sd-LDL-C. During the observation period, 116 cases of CVD, 53 cases of stroke, 36 cases of cerebral infarction, and 63 cases of CAD were reported. As shown in **Table 2**, increasing quartiles of sd-LDL-C were significantly associated with increased risks of CVD (stroke + CAD), stroke, cerebral infarction, and CAD after age and multivariable adjustment. Age and sex-adjusted HRs per 10 mg/dL of sd-LDL-C for CVD, stroke, cerebral infarction, and CAD were 1.21 (95% CI: 1.12-1.31), 1.17 (95% CI: 1.05-1.30), 1.15 (95% CI: 1.00-1.33), and 1.29 (95% CI: 1.14-1.45), respectively. HRs after multivariable adjustment were almost the same. When we analyzed each gender, age-adjusted HRs per 10 mg/dL of sd-LDL-C for CVD, stroke, cerebral infarction, and CAD were significant in women, while those for CVD and CAD were significant in men. HR for CAD of the fourth quartile was almost 4 after age and multivariable adjustment in men.

After putting LDL-C into the multivariable adjusted-models (Model A), sd-LDL-C was still associated with increased risk for CVD, stroke, cerebral infarction, and CAD in the total cohort, for CVD in men, and CVD, stroke, and cerebral infarction in women. After further putting logarithmically transformed triglyceride and HDL-C variables into Model A (Model B), sd-LDL-C was still associated with

Table 1. Baseline characteristics of cardiovascular risk factors according to small dense LDL cholesterol quartiles

	Small dense LDL Cholesterol				*p* value for Trend
	Q1	Q2	Q3	Q4	
Men					
Number of subjects	241	243	242	242	
Small dense LDL, range (mean), mg/dL	6.3-27.8 (21.1)	27.9-38.2 (32.7)	38.3-53.4 (45.3)	53.5-119.6 (67.3)	
Age, year	60.9 ± 13.1	59.7 ± 12.5	59.1 ± 12.3	59.4 ± 11.3	0.421
Body mass index, kg/m^2	21.5 ± 2.5	22.4 ± 2.8	23.4 ± 2.4	24.0 ± 2.7	<0.001
TC, mg/dL	170 ± 25	189 ± 24	199 ± 25	220 ± 27	<0.001
HDL-C, mg/dL	60 ± 15	57 ± 14	51 ± 11	48 ± 11	<0.001
LDL-C, mg/dL	86 ± 20	111 ± 21	124 ± 23	140 ± 26	<0.001
Triglyceride, (median) mg/dL	66	87	112	167	<0.001
Large-LDL-C, mg/dL	65 ± 17	78 ± 21	79 ± 22	72 ± 24	<0.001
Sd-LDL-C/LDL-C ratio	0.25 ± 0.05	0.31 ± 0.07	0.38 ± 0.08	0.50 ± 0.11	<0.001
Blood pressure category, %					0.002
Optimal blood pressure	31	26	25	19	
Normal blood pressure	30	24	19	26	
High-normal blood pressure	16	30	25	29	
Hypertension grade 1-3	19	26	29	28	
Antilipidemic drug use, %	1	4	5	8	0.003
Diabetes, %	3	5	7	9	0.023
Current Smoking, %	44	41	41	44	0.021
Current Drinking, %	66	71	72	74	0.577
Women					
Number of subjects	266	267	266	267	
Small dense LDL, range (mean), mg/dL	7.5-23.9 (18.7)	24.0-33.0 (28.6)	33.1-44.6 (38.5)	44.7-136.6 (59.7)	
Age, year	51.7 ± 13.0	57.3 ± 11.9	60.2 ± 11.2	60.4 ± 9.1	<0.001
Body mass index, kg/m^2	21.0 ± 2.5	21.8 ± 3.2	22.5 ± 3.1	23.2 ± 2.8	<0.001
TC, mg/dL	175 ± 23	200 ± 22	216 ± 25	234 ± 32	<0.001
HDL-C, mg/dL	67 ± 13	64 ± 12	60 ± 13	54 ± 12	<0.001
LDL-C, mg/dL	83 ± 17	109 ± 17	130 ± 18	153 ± 30	<0.001
Triglyceride, (median) mg/dL	61	78	97	140	<0.001
Large-LDL-C, mg/dL	64 ± 14	81 ± 15	92 ± 17	93 ± 25	<0.001
Sd-LDL-C/LDL-C ratio	0.23 ± 0.04	0.27 ± 0.04	0.30 ± 0.05	0.40 ± 0.08	<0.001
Blood pressure category, %					<0.001
Optimal blood pressure	34	27	22	17	
Normal blood pressure	25	24	26	25	
High-normal blood pressure	16	29	20	35	
Hypertension grade 1-3	16	21	31	32	
Antilipidemic drug use, %	4	5	6	12	0.002
Diabetes, %	0	1	3	6	<0.001
Current Smoking, %	13	10	6	7	0.056
Current Drinking, %	34	30	22	23	0.014

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Large LDL-C, LDL-C-Sd-LDL-C. Hypertension was defined as described in methods. Diabetes was defined as fasting serum glucose ≥7.0mmol/L (126 mg/dL), the use of anti-diabetic agents, or both.

Table 2. Age- and multivariable-adjusted hazard ratios and 95% confidence intervals for the incidence of cardiovascular disease according to small dense LDL cholesterol quartiles

	Small dense LDL Cholesterol, mg/dL				
	Q1 (Lower)	Q2	Q3	Q4 (Higher)	per 10 mg/dL
Men and women, range (mean)	6.3-25.5 (19.7)	25.6-35.3 (30.5)	35.4-49.0 (41.4)	49.1-136.6 (63.9)	
Person-years	5,576	5,789	5,527	5,741	
Cardiovascular disease					
Case	21	23	29	43	
Age and sex-adjusted HR	1	0.75 (0.43-1.29)	1.11 (0.68-1.83)	**1.64 (1.04-2.60)**	**1.21 (1.12-1.31)**
Model 1-adjusted HR	1	0.81 (0.45-1.42)	1.08 (0.65-1.81)	1.60 (0.99-2.60)	**1.21 (1.11-1.32)**
Stroke					
Case	14	13	10	16	
Age and sex-adjusted HR	1	0.58 (0.30-1.14)	0.80 (0.43-1.48)	1.21 (0.69-2.12)	**1.17 (1.05-1.30)**
Model 1-adjusted HR	1	0.63 (0.32-1.23)	0.79 (0.41-1.50)	1.19 (0.65-2.16)	**1.18 (1.04-1.33)**
Cerebral infarction					
Case	8	10	6	12	
Age and sex-adjusted HR	1	1.08 (0.45-2.57)	1.14 (0.47-2.73)	1.74 (0.77-3.90)	**1.15 (1.00-1.33)**
Model 1-adjusted HR	1	1.18 (0.48-2.88)	1.16 (0.46-2.89)	1.85 (0.77-4.40)	**1.18 (1.00-1.39)**
Coronary artery disease					
Case	7	10	19	27	
Age and sex-adjusted HR	1	1.36 (0.49-3.77)	2.26 (0.89-5.73)	**3.35 (1.38-8.13)**	**1.29 (1.14-1.45)**
Model 1-adjusted HR	1	1.44 (0.51-4.08)	2.17 (0.83-5.66)	**3.26 (1.29-8.20)**	**1.28 (1.13-1.46)**
Men, range (mean)	6.3-27.8 (21.1)	27.9-38.2 (32.7)	38.3-53.4 (45.3)	53.5-119.6 (67.3)	
Person-years	2,499	2,615	2,519	2,608	
Cardiovascular disease					
Case	19	19	22	36	
Age-adjusted HR	1	1.06 (0.56-2.01)	1.31 (0.70-2.44)	**2.03 (1.16-3.57)**	**1.15 (1.04-1.28)**
Model 1-adjusted HR	1	1.17 (0.61-2.24)	1.36 (0.70-2.62)	**2.12 (1.16-3.86)**	**1.16 (1.04-1.30)**
Stroke					
Case	14	13	10	16	
Age-adjusted HR	1	1.03 (0.48-2.21)	0.87 (0.38-1.99)	1.43 (0.69-2.97)	1.06 (0.92-1.23)
Model 1-adjusted HR	1	1.13 (0.51-2.47)	0.98 (0.40-2.38)	1.55 (0.70-3.41)	1.08 (0.92-1.28)
Cerebral infarction					
Case	8	10	6	12	
Age-adjusted HR	1	1.33 (0.52-3.39)	0.85 (0.29-2.48)	1.81 (0.73-4.48)	1.08 (0.91-1.29)
Model 1-adjusted HR	1	1.43 (0.54-3.78)	0.90 (0.29-2.80)	1.93 (0.70-5.29)	1.10 (0.90-1.36)
Coronary artery disease					
Case	5	6	12	20	
Age-adjusted HR	1	1.24 (0.37-4.07)	2.48 (0.87-7.07)	**3.89 (1.45-10.42)**	**1.27 (1.10-1.47)**
Model 1-adjusted HR	1	1.27 (0.38-4.29)	2.34 (0.78-6.97)	**4.03 (1.42-11.40)**	**1.28 (1.09-1.50)**
Women, range (mean)	7.5-23.9 (18.7)	24.0-33.0 (28.6)	33.1-44.6 (38.5)	44.7-136.6 (59.7)	
Person-years	3,077	3,174	3,008	3,133	
Cardiovascular disease					
Case	7	12	13	23	
Age-adjusted HR	1	1.01 (0.39-2.60)	0.99 (0.39-2.50)	1.73 (0.74-4.06)	**1.31 (1.16-1.47)**
Model 1-adjusted HR	1	1.04 (0.40-2.72)	0.91 (0.35-2.35)	1.52 (0.63-3.68)	**1.29 (1.13-1.48)**
Stroke					
Case	5	8	6	16	
Age-adjusted HR	1	0.95 (0.30-2.94)	0.64 (0.19-2.11)	1.72 (0.62-4.74)	**1.31 (1.13-1.52)**
Model 1-adjusted HR	1	0.98 (0.31-3.14)	0.64 (0.18-2.19)	1.66 (0.58-4.76)	**1.33 (1.12-1.59)**

(Cont Table 2)

	Small dense LDL Cholesterol, mg/dL				
	Q1 (Lower)	Q2	Q3	Q4 (Higher)	per 10 mg/dL
Cerebral infarction					
Case	0	5	4	7	
Age-adjusted HR	1	–	–	–	**1.31 (1.05-1.63)**
Model 1-adjusted HR	1	–	–	–	**1.37 (1.05-1.80)**
Coronary artery disease					
Case	2	4	7	7	
Age-adjusted HR	1	1.22 (0.22-7.76)	1.90 (0.39-9.24)	1.84 (0.38-8.91)	**1.32 (1.08-1.61)**
Model 1-adjusted HR	1	1.27 (0.22-7.33)	1.83 (0.35-9.45)	1.54 (0.30-7.83)	1.23 (0.99-1.53)

Model 1: adjusted for age, (sex), body mass index, smoking, drinking, blood pressure category (optimal, normal, and high-normal blood pressure, hypertension grade 1 and 2 + 3), diabetes, and lipid-lowering drug user
Bold numbers: statistically significant

increased risks of CVD and stroke in the total cohort and in women, but not in men (**Table 3**).

Discussion

This study clearly indicates an increased risk of CVD, stroke, cerebral infarction, and CAD attributed to elevated sd-LDL-C concentrations in a Japanese population without a previous history of CVD. We also showed that HR was significant after multivariable adjustment and by analysis including LDL-C, log-transformed triglyceride, and HDL-C in the same model. Thus, sd-LDL-C measurement with the new test is promising as a new biomarker to predict the risk of CVD.

In addition to traditional risk factors for CVD, such as hypertension, diabetes, and dyslipidemia, other biomarkers are required to better define the risk and refine therapeutic decisions. There is scientific evidence that sd-LDL particles are highly atherogenic and can be a biomarker of CVD[15, 20, 21]. Our data provide additional evidence to show the role of sd-LDL-C as a CVD risk in the general population. Furthermore, measuring sd-LDL-C with this test has an advantage because it is more user-friendly and more applicable than specialized tests such as gradient gel electrophoresis, nuclear magnetic resonance, and gradient ultracentrifugation.

Until now, there have been no target goals of sd-LDL-C to prevent CAD. In this study the HR of the 4th quartile was statistically significant, suggesting that the cutoff of sd-LDL-C is approximately 50 mg/dL, although significance was not obtained in women probably due to the low event rate; therefore, a larger study should be performed to define an appropriate cutoff for sd-LDL-C. Because statins, fibrates, and ezetimibe have been shown to reduce the amount of sd-LDL[25-28], a randomized control study is required to address whether lowering sd-LDL-C to a certain goal by these drugs can prevent the development of CAD.

In this study we found that sd-LDL-C was significantly associated with traditional risk factors, such as hypertension and diabetes. BMI and the prevalence of diabetes increased and HDL-C decreased across the sd-LDL-C quartiles, and more hypertensive subjects were found in second to fourth quartiles than in the first quartile in both genders. We also found that age-adjusted partial correlation coefficients between sd-LDL-C and BMI, log-transformed triglyceride, LDL-C, and HDL-C (Pearson) were 0.305, 0.636, 0.554, and −0.346 ($p<0.0001$), respectively. Thus these data suggest that increased concentrations of sd-LDL-C may be associated with metabolic disorders and that lifestyle modification, such as exercise and weight control, would be effective to reduce sd-LDL in patients with diabetes and metabolic syndrome. Furthermore, we should address whether sd-LDL-C can be used to identify a very high-risk patient with type 2 diabetes, metabolic syndrome, and other metabolic disorders. In contrast to the association with metabolic disorders, an age-related change in sd-LDL-C was found only in women, consistent with the trend of increased atherogenic dyslipidemia in postmenopausal women. Ai *et al.* also found that postmenopausal women had higher levels of sd-LDL-C than premenopausal women in the Framingham Offspring Study[20].

In addition to type 2 diabetes and metabolic syn-

Table 3. Relationship between major lipid variables and cardiovascular disease

	Cardiovascular disase	Stroke	Cerebral infarction	Coronary artery disease
Men and women				
Age and sex-adjusted	**1.21 (1.12-1.31)**	**1.17 (1.05-1.30)**	**1.15 (1.00-1.33)**	**1.29 (1.14-1.45)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.21 (1.11-1.32)**	**1.18 (1.04-1.33)**	**1.18 (1.00-1.39)**	**1.28 (1.13-1.46)**
Model A				
Sd-LDL-C/10 mg/dL	**1.26 (1.11-1.43)**	**1.26 (1.06-1.50)**	**1.29 (1.02-1.62)**	**1.29 (1.07-1.55)**
LDL-C/10 mg/dL	0.96 (0.89-1.04)	0.94 (0.85-1.04)	0.93 (0.81-1.06)	0.99 (0.88-1.11)
Model B				
Sd-LDL-C/10 mg/dL	**1.20 (1.01-1.42)**	**1.35 (1.07-1.71)**	1.31 (0.96-1.78)	1.05 (0.81-1.36)
LDL-C/10 mg/dL	0.98 (0.90-1.06)	0.93 (0.83-1.03)	0.92 (0.80-1.07)	1.05 (0.93-1.19)
ln_TG	1.15 (0.71-1.86)	0.76 (0.40-1.46)	0.86 (0.37-1.96)	1.82 (0.87-3.81)
HDL-C/10 mg/dL	0.94 (0.81-1.08)	1.00 (0.84-1.20)	0.93 (0.73-1.18)	0.80 (0.61-1.04)
Men				
Age-adjusted	**1.15 (1.04-1.28)**	1.06 (0.92-1.23)	1.08 (0.91-1.29)	**1.27 (1.10-1.47)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.16 (1.04-1.30)**	1.08 (0.92-1.28)	1.10 (0.90-1.36)	**1.28 (1.09-1.50)**
Model A				
Sd-LDL-C/10 mg/dL	**1.17 (1.00-1.38)**	1.17 (0.92-1.48)	1.20 (0.90-1.60)	1.18 (0.94-1.48)
LDL-C/10 mg/dL	0.99 (0.89-1.09)	0.94 (0.82-1.08)	0.93 (0.79-1.09)	1.07 (0.93-1.24)
Model B				
Sd-LDL-C/10 mg/dL	1.10 (0.88-1.38)	1.28 (0.92-1.77)	1.28 (0.87-1.90)	0.96 (0.70-1.31)
LDL-C/10 mg/dL	1.01 (0.90-1.13)	0.92 (0.78-1.07)	0.91 (0.76-1.10)	1.14 (0.97-1.33)
ln_TG	1.23 (0.66-2.26)	0.75 (0.32-1.76)	0.86 (0.31-2.38)	1.87 (0.75-4.62)
HDL-C/10 mg/dL	0.96 (0.80-1.14)	1.05 (0.85-1.28)	1.08 (0.94-1.40)	0.72 (0.50-1.03)
Women				
Age-adjusted	**1.31 (1.16-1.47)**	**1.31 (1.13-1.52)**	**1.31 (1.05-1.63)**	**1.32 (1.08-1.61)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.29 (1.13-1.48)**	**1.33 (1.12-1.59)**	**1.37 (1.05-1.80)**	1.23 (0.99-1.53)
Model A				
Sd-LDL-C/10 mg/dL	**1.44 (1.17-1.77)**	**1.48 (1.13-1.94)**	**1.62 (1.08-2.43)**	1.33 (0.94-1.89)
LDL-C/10 mg/dL	0.92 (0.81-1.04)	0.92 (0.79-1.08)	0.88 (0.69-1.11)	0.94 (0.75-1.16)
Model B				
Sd-LDL-C/10 mg/dL	**1.35 (1.03-1.77)**	**1.47 (1.04-2.08)**	1.33 (0.78-2.29)	1.12 (0.70-1.79)
LDL-C/10 mg/dL	0.93 (0.81-1.07)	0.92 (0.78-1.09)	0.92 (0.72-1.19)	0.98 (0.78-1.24)
ln_TG	1.19 (0.53-2.69)	0.91 (0.31-2.68)	0.86 (0.17-4.25)	1.84 (0.47-7.15)
HDL-C/10 mg/dL	0.92 (0.72-1.19)	0.92 (0.67-1.26)	0.56 (0.31-1.00)	0.92 (0.60-1.41)

Multivariable adjusted for age, sex, body mass index, smoking, drinking, blood pressure category (optimal, normal, and high-normal bloodpressure, hypertension grade 1 and 2 + 3), diabetes, and antilipidemic drug user
Model A: sd-LDL-C per 10 mg/dL and LDL-C per 10 mg/dL in the same model
Model B: sd-LDL-C per 10 mg/dL, LDL-C per 10 mg/dL, ln(TG), and HDL-C per 10 mg/dL in the same model
Sd-LDL-C, small dense LDL cholesterol; ln_TG, logarithmical transformed TG
Bold numbers: statistically significant

drome, sd-LDL-C is increased in familial combined hyperlipidemia and postprandial hyperlipidemia[29, 30]. Hirano *et al.* demonstrated that sd-LDL-C determined by this simple precipitation method is useful for screening familial combined hyperlipidemia in large populations[13]. Because the prevalence of familial combined hyperlipidemia is high in the general population and the increase of sd-LDL particles as well as large VLDL particles is a characteristic feature of familial combined hyperlipidemia, this assay would be quite useful for its diagnosis. Although sd-LDL is decreased by lipid-lowering drugs, such as statins and fibrates, the effect of adequate combination therapy on sd-LDL-C has not yet been confirmed; therefore, this assay would be also useful in determining the therapeutic strategy for patients with a high serum level of sd-LDL-C.

There are some limitations in our study. First, we

used plasma stored at −80℃, and there is no guarantee that we would have obtained the same results if we had used fresh serum; however, our results are consistent with those reported by Hirano *et al.*, who measured sd-LDL-C in a Japanese general population with the same method, and comparison studies performed in Japan indicate virtually identical results with the use of fresh vs. frozen plasma for sd-LDL-C[13]. Second, the single measurement of sd-LDL-C at the baseline survey and the fact we did not evaluate the longitudinal trend for each risk factor including lipid-lowering agents may have caused us to underestimate the relationship between these conditions and CAD due to regression dilution bias, although we statistically adjusted for the use of lipid-lowering agents at the baseline survey. Third, serum LDL-C was measured by the direct homogeneous assay, which failed to meet the National Cholesterol Education Program total error goals for diseased individuals, although it met these goals in non-diseased individuals[31]. However, the present study is a cohort study of community-dwelling citizens without a history of CVD. Furthermore, the serum levels of LDL-C determined by direct homogeneous assay are almost consistent with those calculated by the Friedewald formula in a large Japanese cohort.

Conclusions

In this large urban cohort study conducted in Japan, we demonstrated that sd-LDL-C is significantly associated with the development of CVD, providing evidence of sd-LDL-C as an important biomarker to predict CVD. A large intervention study is required to determine the appropriate target level of sd-LDL-C.

Sources of Funding

The present study was supported by the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-4- 5), and also supported by grants-in-aid from the Ministry of Health, Labor and Welfare (H22-Seishu-005).

Disclosures

Dr. Arai has received an unrestricted grant from Denka Seiken. Drs Ito and Minagawa are employees of Denka Seiken. The other authors have no conflicts to declare.

References

1) Okamura T, Kokubo Y, Watanabe M, Higashiyama A, Miyamoto Y, Yoshimasa Y, Okayama A: Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: The Suita study. Atherosclerosis, 2009; 203: 587-592
2) Law MR, Wald NJ: An ecological study of serum cholesterol and ischaemic heart disease between 1950 and 1990. Eur J Clin Nutr, 1994; 48: 305-325
3) Law MR, Wald NJ, Rudnicka AR: Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ, 2003; 326: 1423
4) Prospective Studies C, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R: Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet, 2007; 370: 1829-1839
5) Teramoto T, Sasaki J, Ueshima H, Egusa G, Kinoshita M, Shimamoto K, Daida H, Biro S, Hirobe K, Funahashi T, Yokote K, Yokode M: Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. J Atheroscler Thromb, 2007; 14: 45-50
6) Nakamura H, Arakawa K, Itakura H, Kitabatake A, Goto Y, Toyota T, Nakaya N, Nishimoto S, Muranaka M, Yamamoto A, Mizuno K, Ohashi Y: Primary prevention of cardiovascular disease with pravastatin in Japan (MEGA Study): a prospective randomised controlled trial. Lancet, 2006; 368: 1155-1163
7) Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ: Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med, 2008; 359: 2195-2207
8) Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R: Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet, 2005; 366: 1267-1278
9) Berneis KK, Krauss RM: Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res, 2002; 43: 1363-1379
10) Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM: Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest, 1993; 92: 141-146
11) Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW, D'Agostino RB, Vasan RS, Robins SJ: Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. Circulation, 2006; 113: 20-29
12) Deighan CJ, Caslake MJ, McConnell M, Boulton-Jones JM, Packard CJ: Atherogenic lipoprotein phenotype in end-stage renal failure: origin and extent of small dense

low-density lipoprotein formation. Am J Kidney Dis, 2000; 35: 852-862
13) Hirano T, Nohtomi K, Sato Y, Kamata K, Ito Y: Small dense LDL-cholesterol determined by a simple precipitation assay for screening familial combined hyperlipidemia. Atherosclerosis, 2009; 205: 603-607
14) Hirano T, Ito Y, Koba S, Toyoda M, Ikejiri A, Saegusa H, Yamazaki J, Yoshino G: Clinical significance of small dense low-density lipoprotein cholesterol levels determined by the simple precipitation method. Arterioscler Thromb Vasc Biol, 2004; 24: 558-563
15) St-Pierre AC, Cantin B, Dagenais GR, Mauriege P, Bernard PM, Despres JP, Lamarche B: Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol, 2005; 25: 553-559
16) Swinkels DW, Hak-Lemmers HL, Demacker PN: Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low density lipoprotein subfractions. J Lipid Res, 1987; 28: 1233-1239
17) Nichols AV, Krauss RM, Musliner TA: Nondenaturing polyacrylamide gradient gel electrophoresis. Methods Enzymol, 1986; 128: 417-431
18) Hirano T, Ito Y, Saegusa H, Yoshino G: A novel and simple method for quantification of small, dense LDL. J Lipid Res, 2003; 44: 2193-2201
19) Ito Y, Fujimura M, Ohta M, Hirano T: Development of a homogeneous assay for measurement of small dense LDL cholesterol. Clin Chem, 2011; 57: 57-65
20) Ai M, Otokozawa S, Asztalos BF, Ito Y, Nakajima K, White CC, Cupples LA, Wilson PW, Schaefer EJ: Small dense LDL cholesterol and coronary heart disease: results from the Framingham Offspring Study. Clin Chem, 2010; 56: 967-976
21) Koba S, Yokota Y, Hirano T, Ito Y, Ban Y, Tsunoda F, Sato T, Shoji M, Suzuki H, Geshi E, Kobayashi Y, Katagiri T: Small LDL-cholesterol is superior to LDL-cholesterol for determining severe coronary atherosclerosis. J Atheroscler Thromb, 2008; 15: 250-260
22) Mannami T, Baba S, Ogata J: Strong and significant relationships between aggregation of major coronary risk factors and the acceleration of carotid atherosclerosis in the general population of a Japanese city: the Suita Study. Arch Intern Med, 2000: 160: 2297-2303
23) Tunstall-Pedoe H, Vanuzzo D, Hobbs M, Mahonen M, Cepaitis Z, Kuulasmaa K, Keil U: Estimation of contribution of changes in coronary care to improving survival, event rates, and coronary heart disease mortality across the WHO MONICA Project populations. Lancet, 2000; 355: 688-700
24) Walker AE, Robins M, Weinfeld FD: The National Survey of Stroke. Clinical findings. Stroke, 1981; 12: I13-44
25) Mudd JO, Borlaug BA, Johnston PV, Kral BG, Rouf R, Blumenthal RS, Kwiterovich PO Jr: Beyond low-density lipoprotein cholesterol: defining the role of low-density lipoprotein heterogeneity in coronary artery disease. J Am Coll Cardiol, 2007; 50: 1735-1741
26) Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ: Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation, 2006; 113: 1556-1563
27) Vakkilainen J, Steiner G, Ansquer JC, Aubin F, Rattier S, Foucher C, Hamsten A, Taskinen MR, Group, D: Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease: the Diabetes Atherosclerosis Intervention Study (DAIS). Circulation, 2003; 107: 1733-1737
28) Rizzo M, Rini GB, Spinas GA, Berneis K: The effects of ezetimibe on LDL-cholesterol: quantitative or qualitative changes? Atherosclerosis, 2009; 204: 330-333
29) Veerkamp MJ, de Graaf J, Bredie SJ, Hendriks JC, Demacker PN, Stalenhoef AF: Diagnosis of familial combined hyperlipidemia based on lipid phenotype expression in 32 families: results of a 5-year follow-up study. Arterioscler Thromb Vasc Biol, 2002; 22: 274-282
30) Tsunoda F, Koba S, Hirano T, Ban Y, Iso Y, Suzuki H, Geshi E, Katagiri, T: Association between small dense low-density lipoprotein and postprandial accumulation of triglyceride-rich remnant-like particles in normotriglyceridemic patients with myocardial infarction. Circ J, 2004; 68: 1165-1172
31) Miller WG, Myers GL, Sakurabayashi I, Bachmann LM, Caudill SP, Dziekonski A, Edwards S, Kimberly MM, Korzun WJ, Leary ET, Nakajima K, Nakamura M, Nilsson G, Shamburek RD, Vetrovec GW, Warnick GR, Remaley AT: Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem, 2010; 56: 977-986

Association With Serum Gamma-Glutamyltransferase Levels and Alcohol Consumption on Stroke and Coronary Artery Disease

The Suita Study

Aya Higashiyama, MD, PhD; Ichiro Wakabayashi, MD; Yuu Ono, MD; Makoto Watanabe, MD; Yoshihiro Kokubo, MD; Akira Okayama, MD; Yoshihiro Miyamoto, MD; Tomonori Okamura, MD

Background and Purpose—Light-to-moderate alcohol consumption is associated with reduced risk for cardiovascular disease, whereas high serum γ-glutamyltransferase (GGT) level is associated with cardiovascular disease. However, whether light-to-moderate alcohol drinking is still related to reduced risk of cardiovascular disease irrespective of GGT level is uncertain.

Methods—We performed a 12.5-year cohort study of 2336 men (excluding exdrinkers) who were free from cardiovascular disease. They were classified into 4 groups according to alcohol consumption: never, and current light, moderate, or heavy drinker. The multivariate-adjusted hazard ratios of alcohol consumption for incidence of coronary artery disease, total stroke, and ischemic stroke compared with those of never drinkers were assessed with stratification by GGT median (32 IU/L).

Results—In participants with GGT >32 IU/L, the hazard ratios of all current drinkers for total and ischemic stroke were higher than those of never drinkers. However, in all current drinkers with GGT ≤32 IU/L, the multivariate-adjusted hazard ratios for total and ischemic stroke were lower than in never drinkers.

Conclusions—In men with above GGT median, alcohol drinking even with light-to-moderate consumption could be a risk factor for ischemic stroke. **(*Stroke*. 2011;42:1764-1767.)**

Key Words: cohort ■ epidemiology ■ incidence ■ ischemic stroke

Light-to-moderate alcohol drinking is associated with reduced risk of cardiovascular disease (CVD).[1,2] On the other hand, high serum γ-glutamyltransferase (GGT) level is associated with increased risk of CVD.[3,4] However, it is unclear whether light-to-moderate alcohol drinking is still associated with lower risk of CVD irrespective of GGT level. We performed a cohort study in Japanese men to examine the relationships of alcohol drinking with stroke and coronary artery disease (CAD) events after stratification by GGT level.

Methods

The Suita Study, a cohort study of CVD, was established in 1989. The details of the methods have been described elsewhere.[5] In the present study, 2336 men aged 30 to 79 years who were free from CVD and did not include exdrinkers were followed for 12.5±5.3 years. Disease event included the first stroke or CAD incidence or mortality from stroke or CAD. This study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

In the baseline survey, alcohol consumption was determined by alcohol intake per typical week and calculated as ethanol intake per day. We defined 11.5 g of ethanol (half a *gou*, a traditional Japanese unit) as 1 drink; this is nearly equal to 1 "standard" drink in most countries.[6] Participants were classified into 4 groups according to alcohol consumption.

We calculated the multivariate-adjusted hazard ratios (HRs) and 95% CIs for CAD, total stroke and ischemic stroke in each drinking group compared with never drinkers by Cox proportional hazards model after adjusting for possible confounders. The same statistical models were used after the participants were classified according to GGT median (32 IU/L) at the baseline survey and when never drinkers with GGT ≤32 IU/L were used as a reference in all participants. All statistical analyses were performed by using SPSS statistical software, Version 15.0 J (SPSS, Tokyo, Japan).

Results

The baseline characteristics of the participants are shown in Table 1.

During the follow-up period, 109 CAD and 113 total stroke cases occurred. The multivariate-adjusted HRs for CAD were

Received November 9, 2010; final revision received December 14, 2010; accepted December 21, 2010.

From the Department of Environmental and Preventive Medicine (A.H., I.W.), Hyogo College of Medicine, Nishinomiya, Japan; the Department of Preventive Cardiology (Y.O., M.W., Y.K., Y.M.), National Cerebral and Cardiovascular Center, Suita, Japan; Japan Anti-Tuberculosis Association (A.K.), Tokyo, Japan; and the Department of Preventive Medicine and Public Health (T.O.), School of Medicine, Keio University, Tokyo, Japan.

Correspondence to Aya Higashiyama, MD, PhD, Department of Environmental and Preventive Medicine, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, Japan. E-mail ahigashi@hyo-med.ac.jp

© 2011 American Heart Association, Inc.

***Stroke* is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.110.608307**

Downloaded from stroke.ahajournals.org at National Cardiovascular Center on May 31, 2011

Table 1. Baseline Characteristics of Participants According to Alcohol Consumption: The Suita Study

	Alcohol Consumption				
		Current Drinkers			
	Never	Light (<2.0 Drinks/Day)	Moderate (≥2.0 and <4.0 Drinks/Day)	Heavy (≥4.0 Drinks/Day)	Trend *P*
No. of participants	521	317	796	702	
Age, y*	59±14	58±14	55±13	52±11	<0.001
GGT, IU/L†	22 (3–279)	25 (2–343)	33 (5–521)	56 (10–788)	<0.001
Body mass index, kg/m^2*	22.7±3.2	22.5±3.0	22.8±2.7	23.1±2.8	0.003
Hypertension, %‡§	29.4	32.2	32.2	35.9	0.110
Diabetes, %‡§	4.4	5.4	5.3	6.7	0.364
High-density lipoprotein cholesterol, mmol/L*	1.15±0.30	1.25±0.31	1.28±0.33	1.36±0.36	<0.001
Triglycerides, mmol/L†	1.28	1.22	1.25	1.41	<0.001
Hypercholesterolemia, %‡§	29.9	30.0	28.4	27.6	0.785
Current smoking, %‡	48.2	36.9	48.0	62.0	<0.001

Values are mean±SD unless specified otherwise. Values of GGT and triglycerides are median (range).
GGT indicates γ-glutamyltransferase.
*Analysis of variance.
†Kruskal-Wallis test.
‡Chi-square test.
§Hypertension is systolic/diastolic blood pressures of ≥140/90 mm Hg and/or current medication for hypertension. Diabetes is fasting blood glucose of ≥7 mmol/L and/or current use of insulin or oral medication for diabetes. Hypercholesterolemia is fasting serum total cholesterol of ≥5.69 mmol/L and/or current medication for dyslipidemia.

Table 2. Risk of Alcohol Consumption for Cardiovascular Disease According to GGT Level With 12.5-Year (Mean) Follow-Up: The Suita Study

	Alcohol Consumption			
		Current Drinkers		
	Never	Light (<2.0 Drinks/Day)	Moderate (≥2.0 and <4.0 Drinks/Day)	Heavy (≥4.0 Drinks/Day)
Participants with GGT median (32 IU/L) and below				
No. of participants	392	209	396	172
Person-y	4746	2564	5041	2236
Coronary artery disease				
No. of events	35	14	14	4
Multivariate-adjusted HR (95% CI)*	1.00	0.64 (0.34–1.22)	0.40 (0.21–0.76)	0.31 (0.11–0.89)
Total stroke				
No. of events	24	11	12	7
Multivariate-adjusted HR (95% CI) *	1.00	0.63 (0.30–1.31)	0.43 (0.21–0.88)	0.70 (0.29–1.69)
Ischemic stroke				
No. of events	15	9	7	4
Multivariate-adjusted HR (95% CI)*	1.00	0.80 (0.34–1.89)	0.43 (0.17–1.08)	0.63 (0.20–2.01)
Participants with above GGT median (32 IU/L)				
No. of participants	129	108	400	530
Person-y	1619	1329	4923	6831
Coronary artery disease				
No. of events	8	5	15	14
Multivariate-adjusted HR (95% CI)*	1.00	1.14 (0.37–3.54)	0.81 (0.34–1.97)	0.61 (0.24–1.56)
Total stroke				
No. of events	2	8	26	23
Multivariate-adjusted HR (95% CI)*	1.00	7.66 (1.60–36.60)	6.68 (1.56–28.66)	4.68 (1.07–20.54)
Ischemic stroke				
No. of events	2	6	21	14
Multivariate-adjusted HR (95% CI)*	1.00	5.81 (1.15–29.26)	5.27 (1.21–23.02)	2.54 (0.55–11.70)

*Multivariate-adjusted HR was calculated after adjustment for age, body mass index, high-density lipoprotein cholesterol, triglycerides (log-transformed), presence of hypertension, diabetes and hypercholesterolemia, and smoking status (current or noncurrent).
GGT indicates γ-glutamyltransferase; HR, hazard ratio.

Downloaded from stroke.ahajournals.org at National Cardiovascular Center on May 31, 2011

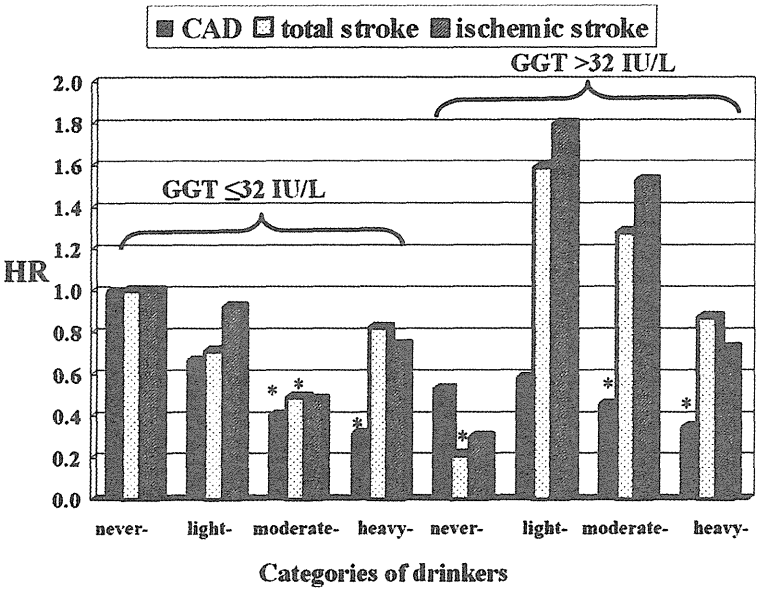


Figure. Risk of alcohol consumption for CVD according to GGT level. *$P<0.05$ compared to never-drinkers with GGT median and below. HR indicates multivariate-adjusted hazard ratio adjusted for the same confounders presented in Table 2; CVD, cardiovascular disease; GGT, γ-glutamyltransferase.

lower in drinkers than in never drinkers with statistical significance in moderate and heavy drinkers. The HRs for total and ischemic stroke were similar among all groups (data not shown).

In participants with GGT ≤32 IU/L, the HRs for CAD, total stroke, and ischemic stroke were lower among all current-drinker groups than in never drinkers (Table 2). In participants with GGT >32 IU/L, HRs for CAD were still lower in moderate and heavy drinkers than in never drinkers. However, for total and ischemic stroke, HRs were higher among current drinkers than in never drinkers. These tendencies did not change when never drinkers with GGT ≤32 IU/L were used as a reference in all participants (Figure), although the HRs were different from those presented in Table 2.

Discussion

To our knowledge, this is the first cohort study to show the consumption-specific risks of alcohol drinking for CVD stratified by GGT levels. Generally, habitual drinkers are interested to know, "What is the upper limit of alcohol intake?" and "Who should not drink?" The answer from our study is "Especially from the viewpoint of stroke prevention, serum GGT levels should be examined; people with high GGT levels should not drink alcohol," although we need to accumulate more evidence concerning this question in future studies.

Increased GGT levels are associated with CVD risk factors such as high blood pressure, high cholesterol, and high blood glucose levels[7] and with high levels of oxidative stress markers.[8] These risk factors might be associated with our current results, although we adjusted for the presence of hypertension, hypercholesterolemia, and diabetes mellitus in the baseline survey.

This study had several limitations. First, because of the small number of cases, we could not assess the risk of hemorrhagic stroke. Second, use of a single GGT measurement and a single assessment of alcohol consumption at the baseline survey might have caused misclassification or underestimation of the relationships among alcohol drinking, GGT, and events because of regression dilution bias.[9] Third, we could not accurately validate our questionnaire for alcohol consumption and investigate the effects of binge drinking, type of alcoholic beverage consumed,[6] and genetic differences.[10] Fourth, the small event number of never drinkers with GGT >32 IU/L was vulnerable to fluctuation and could affect the results.

In conclusion, for individuals with relatively high GGT levels, alcohol drinking could be a risk factor for ischemic stroke. Examination of serum GGT levels might be important for the evaluation of ischemic stroke risk in consumers of alcohol.

Sources of Funding

This study was supported by the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-4-5) and by Grants-in-Aid from the Ministry of Health, Labor, and Welfare (H20-Seishu-013).

Disclosures

None.

References

1. Rimm EB, Williams P, Foscher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *BMJ*. 1999;319:1532–1538.
2. Iso H, Baba S, Mannami T, Sasaki S, Okada K, Konishi M, Tsugane S; for the JPHC Study Group. Alcohol consumption and risk of stroke among middle-aged men: the JPHC Study Cohort I. *Stroke*. 2004;35:1124–1129.
3. Meisinger C, Döring A, Schneider A, Löwel H; for The KORA Study Group. Serum gamma-glutamyltransferase is a predictor of incident coronary events in apparently healthy men from general population. *Atherosclerosis*. 2006;189:297–302.

Downloaded from stroke.ahajournals.org at National Cardiovascular Center on May 31, 2011

4. Shimizu Y, Imano H, Ohira T, Kitamura A, Kiyama M, Okada M, Sato S, Shimamoto T, Yamagishi K, Tanigawa T, Iso H. γ-Glutamyltransferase and incident stroke among Japanese men and women. The Circulatory Risk in Communities Study (CIRCS). *Stroke*. 2010;41:385–388.
5. Kokubo Y, Nakamura S, Okamura T, Yoshimasa Y, Makino H, Watanabe M, Higashiyama A, Kamide K, Kawanishi K, Okayama A, Kawano Y. Relationship between blood pressure category and incidence of stroke and myocardial infarction in an urban Japanese population with and without chronic kidney disease: the Suita Study. *Stroke*. 2009;40:2674–2679.
6. Okamura T, Tanaka T, Yoshita K, Chiba N, Takebayashi T, Kikuchi Y, Tamaki J, Tamura U, Minai J, Kadowaki T, Miura T, Nakagawa H, Tanihara S, Okayama A, Ueshima H; for the HIPOP-OHP Research Group. Specific alcoholic beverage and blood pressure in a middle-aged Japanese population: the High-risk and Population Strategy for Occupational Health Promotion (HIPOP-OHP) Study. *J Hum Hypertens*. 2004; 18:9–16.
7. Sakuta H, Suzuki T, Yasuda H, Ito T. Gamma-glutamyl transferase and metabolic risk factors for cardiovascular disease. *Intern Med*. 2005;44: 538–541.
8. Lee DH, Blomhoff R, Jacobs DR. Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radic Res*. 2004;38:535–539.
9. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease. Part 1, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet*. 1990;335:765–774.
10. Amamoto K, Okamura T, Tamaki S, Kita Y, Tsujita Y, Kadowaki T, Nakamura Y, Ueshima H. Epidemiologic study of the association of low-*Km* mitochondrial acetaldehyde dehydrogenase genotypes with blood pressure level and the prevalence of hypertension in a general population. *Hypertens Res*. 2002;25:857–864.

Downloaded from stroke.ahajournals.org at National Cardiovascular Center on May 31, 2011

5 狭窄・閉塞病変の評価

[redacted]

はじめに

頭部領域の血管エコーには経頭蓋ドプラ法(transcranial Doppler：TCD)と経頭蓋カラードプラ法(transcranial color-coded duplex sonography：TCCD)があり，それぞれに狭窄・閉塞病変の評価に関する報告がある．また，評価血管も側頭骨窓を介した中大脳動脈(middle cerebral artery：MCA)，前大脳動脈(anterior cerebral artery：ACA)，内頸動脈(internal carotid artery：ICA)や，大後頭孔窓を介した椎骨動脈(vertebral artery：VA)，脳底動脈(basilar artery：BA)など多岐にわたる．本稿ではTCD，TCCD両検査のパルスドプラ法から得られる血流所見を中心に主幹脳動脈の狭窄・閉塞病変の評価方法を解説する．

TCDによる評価

TCDはドプラ血流速波形のみを描出する盲目的な検査であり，超音波入射角は調整できないため血流速度の絶対値は計測できない．このため，

表1 TCDにおける主幹脳動脈の狭窄所見

MCA(M1)狭窄 側頭骨窓 深度45～60 mm	• 狭窄部における血流速度の上昇(平均血流速度100 cm/s以上) • 乱流(turbulence wave form：基線に沿った規則的な異常ドプラ信号)の存在 • 同側のACA平均血流速度の上昇(代償的血流増加：compensatory flow diversion) • 特徴的な異常ドプラ音の聴取(low frequency noise) • 狭窄部より遠位部におけるmicroembolic signals(MES)の検出
ACA狭窄 側頭骨窓 深度60～75 mm	• ACAの平均血流速度がMCA平均血流速度より早い • 平均血流速度が80 cm/s以上 • 収縮期血流速度140 cm/s以上
PCA狭窄 側頭骨窓 深度55～65 mm	• 狭窄部位の平均血流速度と正常部位の平均血流速度が30%を超える場合 • 平均血流速度が50 cm/s以上
ICAサイフォン部狭窄 眼窩骨窓 深度60～65 mm	• 平均血流速度65 cm/s以上 • 収縮期血流速度90 cm/s以上
VA狭窄 大後頭骨窓 深度70～75 mm	• 狭窄部位の平均血流速度と正常部位の平均血流速度比が30%を超える場合 • 平均血流速度が50 cm/s以上
BA狭窄 大後頭骨窓 深度80～100 mm	• 狭窄部位の平均血流速度と正常部位の平均血流速度比が30%を超える場合 • 平均血流速度が60 cm/s以上

ACA：anterior cerebral artery, MCA：middle cerebral artery, PCA：posterior cerebral artery, ICA：internal carolid artery, VA：vertebral artery, BA：basilar artery.

血管病変の診断はドプラ血流速波形の変化を丹念に追い，同一血管内での血流波形の変化や，隣接あるいは対側の血流波形と比較することで傍証を積み上げていく．

狭窄所見

狭窄部の診断は，主に目的動脈の平均血流速度や収縮期血流速度の上昇をもって行う（**表1**）[1,2]．TCDが最も活用されているのはMCA水平部（M1）における狭窄部の検出である．側頭骨窓で検出したドプラ血流速波形は深度60～65 mm付近で双方向に描出されるため，ACAとMCAの分岐部が同定できる．同定後に波形を追いながらサンプルボリュームの深度を60～45 mmと徐々に浅くすることで，MCA水平部の血流波形の変化を観察することができる．観察過程で大幅な血流速度の上昇を捉えれば，狭窄部の存在が示唆される．その他にも，乱流所見の存在やACAの血流速度との比較，微小栓子信号の検出なども参考所見となる．

閉塞所見

閉塞所見は血流信号の消失をもって診断するが，盲目的検査であるTCDでは，技術的に血流信号を検出できないのか，真に途絶しているかを確認することは難しい．単に血流信号が捉えられないことだけで閉塞と診断することは早計である．一方，側副血行路となっている主幹脳動脈の血流速度の上昇を間接所見とする診断法がある（**表2**）[1,2]．

TCCDによる評価

TCCDはカラードプラ画像で主幹脳動脈を同定し，その血管のドプラ血流速波形を検出する．TCDと異なり超音波入射角の調整が可能であることから，より正確な血流速度を求めることができる．TCCDで計測される各主幹脳動脈の血流速度は健常者であっても年齢ごとに変化し，おおむね加齢とともに低下する（**表3**）[3]．

表2 TCDにおける主幹脳動脈の閉塞所見

直接所見	• 血流信号が描出不能
間接所見（側副血行路の傍証）	MCA閉塞 • ACAの平均血流速度が同側MCA平均血流速度より10%を超えて上昇 MCAもしくはICA閉塞 • PCA平均血流速度が同側MCA平均血流速度より10%を超えて上昇（ICA閉塞の場合あり）

ACA：anterior cerebral artery, MCA：middle cerebral artery, PCA：posterior cerebral artery.

よくある間違い・注意点

- TCCDにおけるMCA閉塞診断では，超音波造影剤の使用の有無でカットオフ値がやや異なることに注意する．
- 椎骨動脈は低形成と狭窄の鑑別が困難であり，TCDやTCCDによる閉塞診断は確立していない．

表3　健常例における年齢層ごとのTCCD血流速度の変化（文献3より改変）

血流速度 (cm/s)	n数	年齢層 全例	20～40歳	41～60歳	60歳以上
ACA	313				
PSV		79 (37～121)	82 (40～124)	80 (36～124)	72 (52～102)
MV		53 (33～83)	56 (42～84)	53 (37～85)	44 (22～66)
EDV		35 (15～57)	38 (16～60)	35 (13～57)	28 (12～44)
MCA	335				
PSV		110 (54～166)	120 (64～176)	109 (65～175)	92 (58～126)
MV		73 (33～133)	81 (41～121)	73 (35～111)	59 (37～81)
EDV		49 (21～77)	55 (29～81)	49 (23～75)	37 (21～53)
PCA	336				
PSV		71 (39～103)	75 (43～107)	74 (40～108)	62 (38～86)
MV		49 (25～73)	52 (28～76)	51 (25～75)	40 (22～58)
EDV		33 (15～51)	36 (20～52)	49 (23～75)	26 (14～38)

ACA：anterior cerebral artery, MCA：middle cerebral artery, PCA：posterior cerebral artery, PSV：peak systolic velocity, MV：mean velocity, EDV：end diastolic velocity.

表4　TCCDと脳血管造影の比較（文献4より改変）

	TCCD					血管造影
	PSV カットオフ値 (cm/s)	感度 (%)	特異度 (%)	陽性的中率 (ppv)	陰性的中率 (ppv)	n数	平均狭窄率 (%)
≧50%狭窄の有無							
ACA	≧155	100	100	100	100	4	60±8 (52～71)
MCA	≧220	100	100	100	100	11	67±11 (50～80)
PCA	≧145	100	100	100	91	10	63±7 (50～72)
BA	≧140	100	100	100	100	3	67±14 (53～85)
VA	≧120	100	100	100	100	3	69±14 (55～84)
＜50%狭窄の有無							
ACA	≧120	100	99	73	100	5	38±12 (20～47)
MCA	≧155	94	100	95	100	18	36±8 (22～48)
PCA	≧100	100	100	100	91	5	29±12 (13～41)
BA	≧100	100	100	100	100	4	33±4 (29～37)
VA	≧90	100	100	100	100	5	32±6 (25～39)

ACA：anterior cerebral artery, MCA：middle cerebral artery, PCA：posterior cerebral artery, VA：vertebral artery, BA：basilar artery.

表5 TCCDにおける主幹脳動脈の狭窄所見（国内報告）

MCA（M1）狭窄 側頭骨窓	• 収縮期血流速度 180 cm/s 以上 • 収縮期血流速度 170 cm/s 以上（超音波造影剤使用下）
BA 狭窄 大後頭骨窓	• 収縮期血流速度 120 cm/s 以上（超音波造影剤使用下）

MCA：middle cerebral artery, BA：basilar artery.

表6 TCCDにおける主幹脳動脈の閉塞所見

MCA（M1）閉塞 側頭骨窓	• 拡張末期血流速度が 25 cm/s 以下で MCA 拡張末期血流速度比が 2.7 以上（**図1** 参照） • 収縮期血流速度が 170 cm/s 以下かつ拡張末期血流速度が 26 cm/s 以下であり，MCA 拡張末期血流速度比が 2.6 以上（超音波造影剤使用下，**図2** 参照） • 血流信号は描出不能（超音波造影剤使用下）
BA 閉塞 大後頭骨窓	• 血流信号は描出不能（超音波造影剤使用下） • 逆流所見の存在

MCA：middle cerebral artery, BA：basilar artery.

狭窄部の診断

主幹脳動脈の診断は，TCD と同様に目的血管の血流速度の上昇を捉えることで診断する．既報では TCCD と血管造影検査の比較による狭窄部診断のカットオフ値が示されており，狭窄度が高いほどカットオフ値も上昇する（**表4**）[4]．また，もともとの血流速度が他の主幹脳動脈より速い MCA では，カットオフ値がより高く設定されている．VA では収縮期血流速度が 30 cm/s 以下であるか，健常側より 50％低下する場合は高度狭窄病変と診断できる[5]．国内報告では，MCA（M1）狭窄のカットオフ値は，通常の TCCD では収縮期血流速度 180 cm/s 以上，超音波造影剤の使用下では収縮期血流速度 170 cm/s 以上で診断する（**表5**）[1, 2, 6]．BA 狭窄は超音波造影剤の使用下では収縮期血流速度 120 cm/s 以上で診断する[7]．

閉塞部の診断

MCA（M1）閉塞は超音波造影剤を使用しても血流信号が描出できない場合，もしくは著しく弱い場合に診断される（**表6**）[2]．また，M1 閉塞は MCA 拡張末期血流速度比（病側 MCA 拡張末期血流速度/健常側 MCA 拡張末期血流速度）でも診断され，通常は拡張末期血流速度が 25 cm/s 以下で MCA 拡張末期血流速度比が 2.7 以上であれば M1 閉塞と診断される（**図1, 2**）[6]．超音波造影剤を使用した場合は，収縮期血流速度が

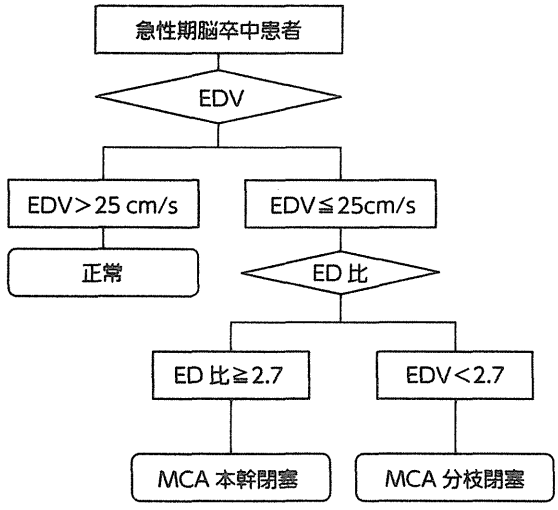
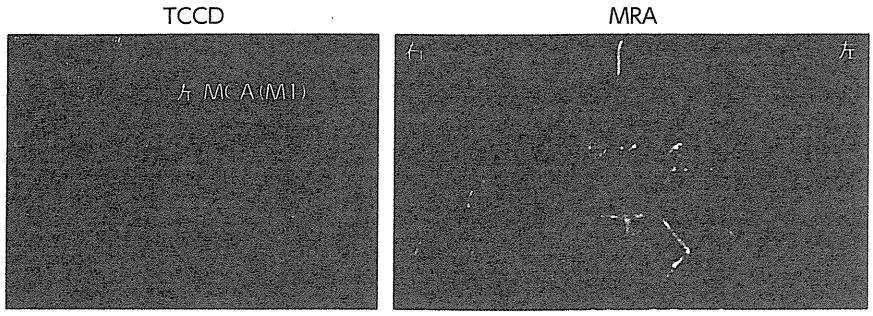


図1 MCA拡張末期血流速度比を用いたMCA閉塞診断
（文献5より改変）

MCA：middle cerebral artery, EDV：end diastolic velocity.



血流速度

	PSV	EDV	MV
左 MCA（M1）	24.8 cm/s	12.3 cm/s	16.0 cm/s
右 MCA（M1）	182.6 cm/s	72.0 cm/s	115.7 cm/s

MCA：middle cerebral artery, ACA：anterior cerebral artery, PSV：peak systolic velocity, EDV：end diastolic velocity, MV：mean velocity.

図2　左MCA閉塞例

左 MCA の拡張末期血流速度は 25 cm/s 以下で，MCA 拡張末期血流速度比（ED ratio）は 5.9 であることから，MCA 閉塞と診断した．頭部 MRA 所見では leptomeningeal anastomosis を中心とした側副血行路が発達している．右 MCA に高度狭窄は認めなかった．

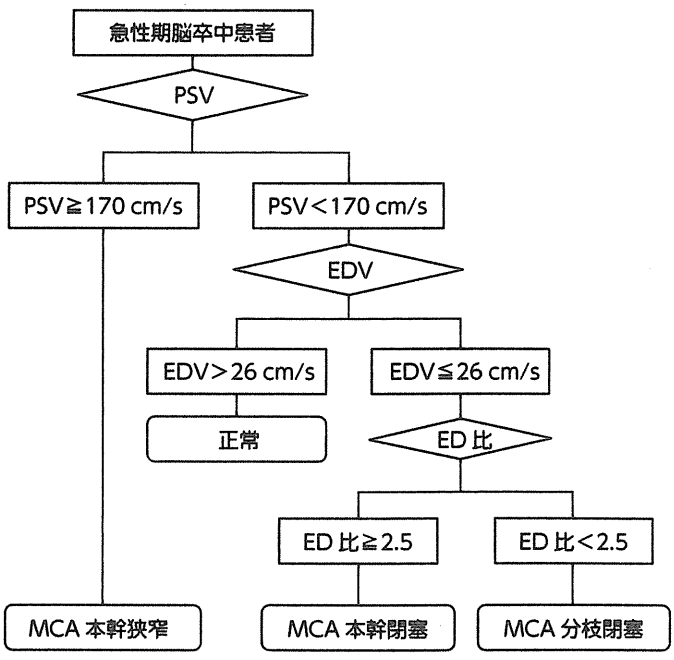


図3　超音波造影剤投与下におけるMCA拡張末期血流速度比を用いたMCA狭窄・閉塞診断
（文献7より改変）

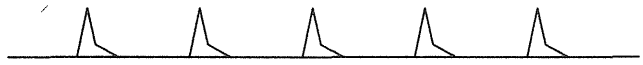
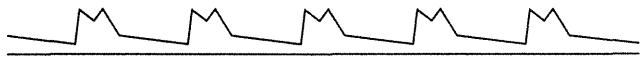
MCA：middle cerebral artery, PSV：peak systolic velocity, EDV：end diastolic velocity.

170 cm/s 以下かつ拡張末期血流速度が 26 cm/s 以下であり，MCA 拡張末期血流速度比が 2.6 以上であれば M1 閉塞と診断される（図3）[8]．BA 閉塞の場合は超音波造影剤を用いても血流信号は描出できないか，逆流所見を認める [9]．

治療効果の判定

TCCD のドプラ血流速波形の変化は，血管内治療や血管手術前後の効果判定に用いるための評価基準が定められている（表7）[10]．この中では，1 段階以上のグレード上昇や正常波形への復帰が再灌流所見とされ，グレードが留まれば変化なし，1

表7 consensus on grading intracranial flow obstruction (COGIF) score（文献9より改変）

グレード	血流パターン	ドプラ血流速波形の模式図
1	血流信号なし	
2	拡張期波形を伴わない血流速度の低下	
3	拡張期波形を伴う血流速度の低下	
4 正常な血流波形	a）対側と等しい血流速度	
	b）局所的な血流速度の上昇	
	c）全般的な血流速度の上昇	

描出・読影のコツ

狭窄・閉塞を有する動脈は血流信号が弱いため，検査開始時はパルストプラ法のサンプルボリュームを大きく広げ，血管の存在が予想される部位を丹念に検索する．サンプルボリュームは血流信号を検出してから徐々に絞り込む．

覚え方のコツ

- TCD における狭窄診断のカットオフ値は平均血流速度が中心であり，ICA 系が VA・BA 系よりも高い．まずは MCA 100 cm/s 以上，VA 50 cm/s 以上を目安に覚える．
- TCCD では，まず M1 狭窄診断のカットオフ値である収縮期血流速度 180 cm/s 以上を覚え，MCA 拡張末期血流速度の極端な左右差を見逃さないよう留意する．

段階以上のグレード低下は悪化と定義されている．また局所的な血流上昇では狭窄を，目的血管全域にわたる血流速度の上昇では過灌流を想定する．

おわりに

TCD や TCCD を用いた狭窄・閉塞診断は，脳卒中超急性期での利用が期待されている．しかし時間制限の厳しい超急性期に短時間で TCD や TCCD を実施するためには，日常的に検査に慣れておく必要がある．近年は TCCD を搭載した超音波機器が増えており，プログラムの切り替え時間も短くなっている．普段の頸動脈エコー検査で少しでも頭蓋内病変を疑った場合は，直ちに TCD や TCCD を実施し，検査の習熟に務めることが望ましい．

参考文献

1) 日本脳神経超音波学会・栓子検出と治療学会合同ガイドライン作成委員会．頭蓋内超音波検査ガイドライン．Neurosonology. 19, 2006, 113-31.
2) 井口保之ほか．経頭蓋ドプラ法・経頭蓋カラードプラ断層法．神経内科．69, 2008, 413-21.
3) Krejza, J. et al. Transcranial Color Doppler Sonography of Basal Cerebral Arteries in 182 Healthy Subjects : Age and Sex Variability and Normal Reference Values for Blood Flow Parameters. AJR. 172, 1999, 213-8.
4) Baumgartner, RW. et al. Assessment of ＞50% and ＜50% Intracranial Stenoses by Transcranial Color-Coded Duplex Sonography. Stroke. 30, 1999, 87-92.
5) Tian, J. et al. Transcranial color Doppler flow imaging in detecting severe stenosis of the intracranial vertebral artery : a prospective study. Clin Imaging. 30, 2006, 1-5.
6) Ogata, T. et al. Transcranial Color-Coded Real-Time Sonographic Criteria for Occlusion of the Middle Cerebral Artery in Acute Ischemic Stroke. AJNR Am J Neuroradiol. 25, 2004, 1680-4.
7) Tateishi, Y. et al. Contrast-Enhanced Transcranial Color-Coded Duplex Sonography Criteria for Basilar Artery Stenosis. J Neuroimaging. 18, 2008, 407-10.
8) Ogata, T. et al. Diagnosis of middle cerebral artery occlusive lesions with contrast-enhanced transcranial color-coded real-time sonography in acute stroke. Neuroradiology. 47, 2005, 256-62.
9) Koga, M. et al. Relationship between Findings of Conventional and Contrast-Enhanced Transcranial Color-Coded Real-Time Sonography and Angiography in Patients with Basilar Artery Occlusion. AJNR Am J Neuroradiol. 23, 2002, 568-71.
10) Nedelmann, M. et al. Consensus Recommendations for Transcranial Color-Coded Duplex Sonography for the Assessment of Intracranial Arteries in Clinical Trials on Acute Stroke. Stroke. 40, 2009, 3238-44.