

Original Paper

Higher Levels of Cystatin C Are Associated with Extracranial Carotid Artery Steno-Occlusive Disease in Patients with Noncardioembolic Ischemic Stroke

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Key Words

Cystatin C · Chronic kidney disease · Carotid artery stenosis · Ischemic stroke

Abstract

Background: Large artery atherosclerosis is a major cause of ischemic stroke worldwide. Differential biomarker profiles associated with extra- and intracranial atherosclerosis are a topic of considerable interest. Cystatin C (CysC), a marker of renal function, is a risk factor for cardiovascular disease. **Aim:** We sought to determine whether CysC levels were associated with extra- and intracranial large artery stenosis (LAS) in patients with acute ischemic stroke. **Methods:** We retrospectively analyzed data of acute noncardioembolic ischemic stroke patients who were admitted to our stroke center within 5 days from symptom onset. Serum CysC levels were measured using latex agglutination turbidimetric immunoassay. Extra- and intracranial LAS were defined as $\geq 50\%$ diameter stenosis or occlusion of the relevant internal carotid artery (ICA) and/or middle cerebral artery (MCA) using carotid echography and volume rendering on magnetic resonance angiography. Multivariate logistic analyses were used to assess the association between CysC levels and LAS after adjustment for potential confounders. **Results:** Of 205 patients (mean age 70.2 years), 76 (37.1%) had LAS. The distribution of LAS was 29 extracranial ICA, 34 intracranial ICA/MCA (8 ICA only, 25 MCA only, 1 ICA+MCA) and 13 tandem stenosis (both extracranial ICA and intracranial ICA/MCA). Levels of CysC were higher in patients with extracranial ICA stenosis than in those with intracranial ICA/MCA stenosis (1.23 ± 0.33 vs. 0.97 ± 0.21 mg/l, $p < 0.001$). In multivariate analysis, the highest CysC tertile (>1.04 mg/l) was significantly associated with extracranial ICA stenosis (adjusted odds ratio [OR] 5.01, 95% confidence interval [CI] 1.51–16.63, $p = 0.009$) after adjustment for age, sex,

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diabetes, chronic kidney disease, current smoking, systolic blood pressure, HDL cholesterol, high-sensitivity C-reactive protein (hs-CRP) and pre-morbid lipid-lowering drugs use. When CysC was considered as a continuous variable, 1 SD increase in CysC was significantly associated with extracranial ICA stenosis (adjusted OR 3.01, 95% CI 1.58–5.72, $p = 0.001$). However, there were no significant associations between CysC levels and intracranial ICA/MCA stenosis. In addition, CysC levels showed a weak but statistically significant correlation with hs-CRP levels ($r = 0.195$, $p = 0.021$). Using receiver operating characteristic curve analysis, CysC value displayed good performance in discriminating extracranial ICA stenosis (c -statistic 0.79, 95% CI 0.69–0.89, $p < 0.001$). **Conclusions:** This preliminary study suggests that higher levels of CysC were independently associated with symptomatic extracranial ICA stenosis, but not with intracranial ICA/MCA stenosis in patients with noncardioembolic stroke. Our findings provide new insights into the link between serum CysC and carotid atherosclerosis.

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Introduction

Extra- and intracranial large artery atherosclerosis is a major cause of ischemic stroke worldwide. Differences in mechanisms underlying extra- and intracranial atherosclerosis are a fascinating topic. Vascular risk factors and biomarker profiles associated with asymptomatic cerebral atherosclerosis have been suggested to be dissociated in extra- versus intracranial large arteries [1]. In addition, it has been noted that intracranial large artery stenosis (LAS) is more common in Asian than in Western populations. Recently, the Chinese Intracranial Atherosclerosis Study has demonstrated that the prevalence of intracranial large artery steno-occlusive disease was 46.6% in patients with acute cerebral ischemia [2]. The Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) trial showed that there was a higher rate of history of diabetes in patients with internal carotid artery (ICA) stenosis than with middle cerebral artery (MCA) stenosis [3]. In addition, previous studies have shown that adiponectin, insulin resistance and high-sensitivity C-reactive protein (hs-CRP) were associated with intracranial atherosclerosis in patients with ischemic stroke [4–6]. In particular, hs-CRP is an inflammatory marker known to be associated with the pathogenesis of atherosclerosis and the development of cardiovascular events. Previous studies have also shown an association between elevated CRP levels and carotid artery stenosis [7, 8]. However, studies comparing hs-CRP levels between symptomatic and asymptomatic carotid artery stenosis have produced conflicting results [9, 10].

Cystatin C (CysC), a protein with a low molecular weight of 13 kDa, is a cysteine proteinase inhibitor. CysC is a serum measure of renal function and has been reported to be a risk factor for cardiovascular events and death [11]. In addition, CysC has attracted attention because it has been found to be an independent risk factor for cardiovascular disease and all-cause mortality in elderly patients with a normal estimated glomerular filtration rate (eGFR) [12]. Furthermore, it has been suggested that CysC concentrations are directly related to both inflammation and atherosclerosis [13], and it could possibly be used as an early predictive factor for the incidence of cardiovascular disease. In patients with asymptomatic carotid atherosclerosis, an association between CysC and the risk of cardiovascular events has been observed [14].

However, no data are available on the association between kidney disease markers and extra- or intracranial LAS. We hypothesized that serum CysC levels are associated with LAS and that CysC would be differentially associated with extra- versus intracranial carotid arterial stenosis. Therefore, the main purpose of the present study was to clarify the impact of CysC levels on extra- or intracranial LAS among patients with acute ischemic stroke.

Subjects and Methods

Study Population

We retrospectively reviewed data from a prospective stroke database for patients who were admitted to our stroke center within 5 days from symptom onset between May 2012 and December 2014. Patients were excluded from the study if they met the following criteria: potential cardiac embolic sources (e.g. atrial fibrillation, dilated cardiomyopathy or valvular heart disease), severe renal dysfunction (creatinine ≥ 1.5 mg/dl), active cancer, chronic inflammatory disease such as collagen disease, and missing data for urinary measurement. Patients with posterior circulation ischemic stroke were excluded from the study because of the small sample size for vertebral-basilar artery stenosis. Finally, 205 patients were included in this study. According to the location of LAS, patients were categorized into four groups: (1) those with extracranial ICA stenosis, (2) those with intracranial ICA/MCA stenosis, (3) those with tandem stenosis (both extracranial ICA and intracranial ICA/MCA) and (4) those without LAS. The categories of intracranial ICA stenosis and MCA stenosis were combined because of the limited number of patients with intracranial ICA stenosis only. Patient selection and grouping is provided in online supplementary figure 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000443338).

Measurement of Kidney Disease Markers

All of the blood samples were drawn at the time of admission or the first morning after admission. Serum CysC levels were measured using a latex agglutination turbidimetric immunoassay (Auto Cystatin C; BML Inc., Tokyo, Japan). The range of the assay is 0.1–14.0 mg/l, with the reference range for healthy individuals reported as 0.40–0.91 mg/l. The intra- and inter-assay coefficient of variation ranged from 0.48 to 1.27% and from 0.55 to 0.81%, respectively. Albuminuria was determined based on the urinary albumin-creatinine ratio (UACR) obtained from the spot urine analysis on the first morning after admission. The UACR was calculated from the urinary albumin, which was estimated using the latex agglutination method and urinary creatinine concentration, as described previously [15]. Each patient's eGFR was calculated using the following three-variable Japanese equation: $eGFR (\text{ml/min}/1.73 \text{ m}^2) = 194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} \times 0.739$ (if female) [16]. Serum creatinine levels were measured using an automated enzymatic method.

Traditional Vascular Risk Factors

Hypertension was defined as previously diagnosed and undergoing therapy with antihypertensive drugs at the time of admission or both blood pressures exceeding 140/90 mm Hg for three measurements. Diabetes was defined as currently undergoing diabetic therapy or fasting blood glucose ≥ 126 mg/dl or HbA1c $\geq 6.5\%$ (National Glycohemoglobin Standardization Program). Dyslipidemia was defined as taking lipid-lowering drugs or one or more of the following: LDL cholesterol ≥ 140 mg/dl, HDL cholesterol < 40 mg/dl or triglycerides ≥ 150 mg/dl. Chronic kidney disease (CKD) was defined as the presence of albuminuria (UACR ≥ 30 mg/g creatinine) and/or a low eGFR (< 60 ml/min/1.73 m²). Smoking status was defined as current use. Atrial fibrillation was diagnosed based on at least one ECG before or during hospitalization.

Measurement of hs-CRP

Blood was immediately separated by centrifugation at 3,000 rpm at 4°C for 10 min after admission and stored at -80°C until analysis. Measurements of hs-CRP were carried out from frozen serum samples with the use of a latex nephelometry method (BN II, Dade Behring Inc., Marburg, Germany).

Evaluation of Extra- and Intracranial LAS

B-mode color Doppler carotid echography (Prosound α 10; Hitachi-Aloka Medical, Tokyo, Japan) was performed on all of the stroke patients within the first 7 days after admission. Extracranial LAS was defined as a narrowing of the relevant ICA lumen of $\geq 50\%$ or occlusion on carotid echography (fig. 1a). The degree of carotid stenosis on duplex ultrasonography was calculated using the criteria based on the European Carotid Surgery Trial (ECST) [17]. All of the magnetic resonance imaging (MRI) examinations were performed on a Signa Horizon 1.5T instrument (GE Healthcare, Milwaukee, Wis., USA). The MRI protocol for acute stroke in our hospital included four sequences: diffusion-weighted imaging, fluid-attenuated inversion-recovery, T2*-weighted gradient echo and intracranial time-of-flight magnetic resonance angiography (MRA). All of the patients underwent initial MRI scans at admission and follow-up MRI within 7 days after admission. The common MRA parameters included a flip angle of 20° , a matrix number of 288×160 and a field of view of 200 mm. Intracranial LAS was defined as $\geq 50\%$ diameter stenosis or occlusion in the relevant MCA trunk or ICA siphon using volume rendering on MRA based on the method from the WASID trial (fig. 1b, c) [18]. Presence of tandem stenosis was defined as $\geq 50\%$ stenosis in both extracranial ICA and intracranial ICA/MCA, including symptomatic lesions in the same vascular territories. A trained radiologist and a trained ultrasonographer, both blinded to the clinical information, assessed the degree of LAS on the carotid echography and MRA.

Statistical Analyses

The patients were subdivided into tertiles according to CysC levels. We compared the clinical characteristics of patients across the tertile groups. To identify the potential variables associated with LAS, the clinical characteristics were compared between patients with and without LAS. Parametric and nonparametric comparisons of categorical and continuous variables were performed using the χ^2 test, the unpaired t test and the Mann-Whitney U test, as appropriate. The hs-CRP levels were analyzed as log-transformed values because their distributions were highly skewed. We performed a multivariate logistic regression analysis to determine whether serum CysC and hs-CRP levels were independent predictors for extracranial ICA or intracranial ICA/MCA stenosis. Variables that indicated a univariate relationship with any LAS ($p < 0.05$) were entered into the multivariate model. If there were strong collinearity characteristics between two variables, we deleted either variable from the independent variables in the multiple regression model. The discriminative ability of CysC and hs-CRP values and extracranial or intracranial LAS was evaluated using receiver operating characteristic curve analysis. A two-sided p value < 0.05 was considered statistically significant. All of the statistical analyses were performed using the SPSS 21.0 software (IBM SPSS, Chicago, Ill., USA).

Results

Patient Characteristics

In a total of 205 patients, the mean age was 70.2 ± 10.9 years, and 134 patients (65.4%) were men. The mean CysC level was 0.97 ± 0.24 mg/l, and median (interquartile range) was 0.95 (0.80–1.09) mg/l. Across the CysC tertile levels, a higher level of CysC was associated with older age, male sex, CKD, current smoking, a higher level of hs-CRP, creatinine or UACR, and a lower eGFR level, as shown in table 1. Of the 205 patients, 76 (37.1%) had LAS. The distribution of LAS was 29 extracranial ICA, 34 intracranial ICA/MCA (8 ICA only, 25 MCA only, 1 ICA+MCA) and 13 tandem stenosis (both extracranial ICA and intracranial ICA/MCA). Regarding kidney biomarkers, patients with extracranial ICA stenosis were more likely to have higher levels of CysC and creatinine and a lower eGFR compared with patients without

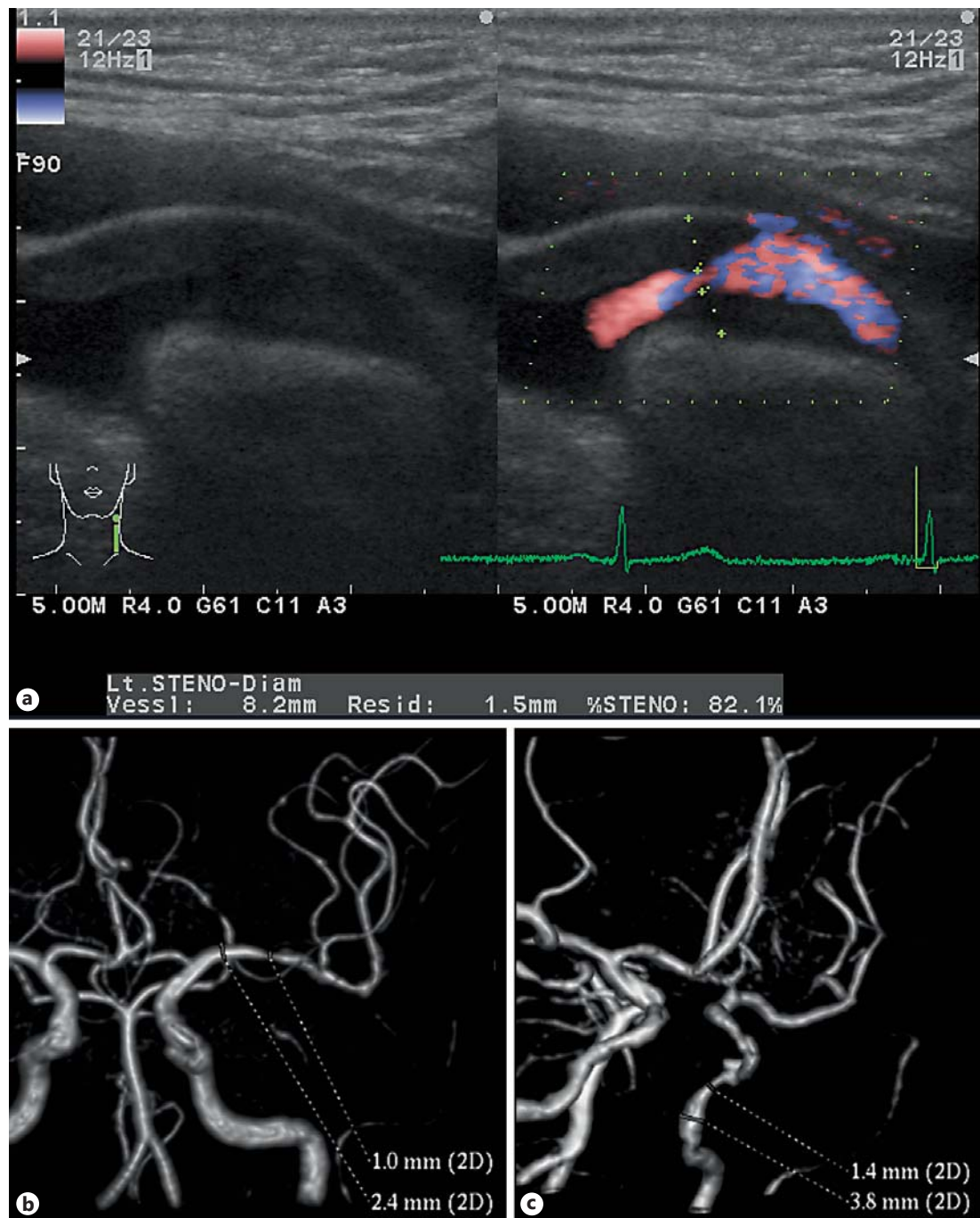


Fig. 1. Examples of extra- and intracranial LAS. **a** Extracranial LAS was defined as ICA stenosis $\geq 50\%$ or occlusion using the ECST method on carotid echography. **b, c** Intracranial LAS was defined as a focal narrowing $\geq 50\%$ in luminal reduction or occlusion in the MCA trunk or ICA siphon using volume rendering on MRA based on the WASID method.

LAS. There were no significant differences in UACR levels between patients with and without LAS. Compared with patients without LAS, patients with extracranial ICA stenosis were older, more often male and more likely to have a history of CKD and premorbid lipid-lowering drugs use, and to have higher hs-CRP levels, lower diastolic blood pressure and lower HDL choles-

Table 1. Comparison of clinical characteristics across the CysC tertile levels

Variables	CysC tertile			p for trend
	lowest (n = 68)	middle (n = 68)	highest (n = 69)	
Age, years	66.3±11.1	70.2±9.2	74.0±11.2	<0.001
Male sex	31 (45.6%)	50 (73.5%)	53 (76.8%)	<0.001
Hypertension	43 (63.2%)	52 (77.6%)	52 (75.4%)	0.117
Diabetes	24 (35.3%)	19 (27.9%)	33 (47.8%)	0.126
Dyslipidemia	24 (35.3%)	18 (26.5%)	22 (31.9%)	0.672
CKD	28 (41.2%)	36 (53.7%)	55 (79.7%)	<0.001
Current smoking	23 (33.8%)	36 (52.9%)	42 (60.9%)	0.002
Systolic blood pressure, mm Hg	157.7±30.5	160.1±30.5	147.6±26.2	0.052
Diastolic blood pressure, mm Hg	86.0±16.3	90.5±20.8	80.2±18.1	0.075
Blood glucose, mg/dl	129.5±36.7	129.8±46.0	145.3±77.0	0.103
HbA1c (NGSP), %	6.3±1.1	6.2±1.2	6.4±1.8	0.639
hs-CRP, mg/l	0.83 (0.38–2.55)	1.10 (0.57–2.28)	1.30 (0.70–4.35)	0.046
Creatinine, mg/dl	0.65±0.14	0.83±0.21	0.97±0.19	<0.001
eGFR, ml/min/1.73 m ²	81.8±13.6	69.1±14.5	56.9±12.5	<0.001
UACR, mg/g creatinine	20 (7–62)	17 (7–43)	36 (8–154)	0.013

Values are mean ± SD, n (%) or median (interquartile range 25–75%).

CysC tertiles: lowest <0.84 mg/l, middle 0.84–1.04 mg/l, highest >1.04 mg/l.

NGSP = National Glycohemoglobin Standardization Program.

terol levels. Patients with intracranial ICA/MCA stenosis were more often male and more likely to have a history of diabetes compared with patients without LAS. Levels of CysC were higher in patients with extracranial ICA stenosis than in those with intracranial ICA/MCA stenosis (1.23 ± 0.33 vs. 0.97 ± 0.21 mg/l, $p < 0.001$) (table 2). In addition, CysC levels showed a weak but statistically significant correlation with hs-CRP levels ($r = 0.195$, $p = 0.021$).

Association of CysC and hs-CRP Levels with Extra- and Intracranial LAS

Across the CysC tertile levels, there was a trend in the rate of extracranial ICA stenosis (p for trend <0.001), but not of intracranial ICA/MCA stenosis (fig. 2). Similarly, for hs-CRP tertiles, there was a significant trend in the rate of extracranial ICA stenosis alone (p for trend <0.01). The highest CysC tertile (>1.04 mg/l) was significantly associated with extracranial ICA stenosis (unadjusted odds ratio [OR] 9.05, 95% confidence interval [CI] 3.63–22.56, $p < 0.0001$). This association remained significant even after adjustment for age, sex, diabetes, CKD, current smoking, systolic blood pressure, HDL cholesterol, hs-CRP and pre-morbid lipid-lowering drugs use (adjusted OR 5.01, 95% CI 1.51–16.63, $p = 0.009$). When the CysC level was considered as a continuous variable, the adjusted OR (95% CI) for extracranial ICA stenosis was 3.01 (1.58–5.72, $p = 0.001$) for 1 SD increase in CysC (table 3). Regarding adjustment variables, only male sex was independently associated with extracranial ICA stenosis (OR 6.90, 95% CI 1.31–36.28, $p = 0.023$). However, hs-CRP levels were not significantly associated with extracranial ICA stenosis after adjustment for potential confounding factors (see online suppl. table 1). In addition, there were no significant associations between CysC and hs-CRP levels and intracranial ICA/MCA stenosis.

Predictive Value of CysC and hs-CRP for Extracranial ICA Stenosis

A receiver operating characteristic curve analysis was performed to validate the diagnostic accuracy of CysC and hs-CRP for extracranial ICA stenosis. At a 1.06 mg/l CysC cut-off

Table 2. Clinical characteristics of patients according to location of LAS

Variables	All (n = 205)	Without LAS (n = 129)	E-ICA (n = 29)	I-ICA/MCA (n = 34)	E-LAS + I-LAS ¹ (n = 13)
Age, years	70.2±10.9	68.9±10.8	74.1±9.2 ^c	70.5±10.4	72.9±13.5
Male sex	134 (65.4%)	74 (57.4%)	24 (82.8%) ^a	26 (76.5%) ^a	10 (76.9%) ^a
Hypertension	147 (71.7%)	95 (73.6%)	19 (65.5%)	24 (70.6%)	9 (69.2%)
Diabetes	76 (37.1%)	41 (31.8%)	10 (34.5%)	18 (52.9%) ^a	10 (76.9%) ^b
Dyslipidemia	64 (31.2%)	37 (28.7%)	12 (41.3%)	9 (26.5%)	6 (46.2%)
CKD	119 (58.0%)	70 (54.3%)	22 (75.9%) ^a	19 (55.9%)	8 (61.5%)
Current smoking	101 (49.3%)	57 (44.2%)	14 (48.3%)	20 (58.8%)	10 (76.9%) ^a
Systolic blood pressure, mm Hg	155.0±29.4	159.1±29.6	148.1±28.7	153.3±27.7	131.8±24.1 ^b
Diastolic blood pressure, mm Hg	85.5±18.9	88.6±20.2	79.9±16.8 ^a	83.5±13.6	71.0±11.3 ^b
Blood glucose, mg/dl	135.0±56.5	131.2±44.5	151.9±101.8	137.2±44.3	128.0±43.7
HbA1c (NGSP), %	6.3±1.4	6.2±1.4	6.3±1.7	6.4±1.0	6.8±1.5
HDL cholesterol, mg/dl	50.3±13.8	52.3±14.8	44.1±9.4 ^b	48.1±12.4	52.8±12.4
LDL cholesterol, mg/dl	124.9±35.9	122.9±34.6	128.9±43.5	131.4±32.8	115.0±39.8
hs-CRP, mg/l	1.10 (0.50–2.78)	0.94 (0.50–2.05)	1.70 (1.00–4.82) ^a	1.16 (0.59–5.27)	2.00 (0.37–5.00)
Creatinine, mg/dl	0.82±0.23	0.77±0.22	0.97±0.23 ^{c,e}	0.85±0.18	0.88±0.21
eGFR, ml/min/1.73 m ²	69.2±17.0	72.3±17.2	60.1±17.7 ^c	66.9±11.7	64.8±17.5
UACR, mg/g creatinine	20 (7–69)	23 (9–78)	28 (9–52)	13 (6–58)	20 (8–56)
CysC, mg/l	0.97±0.24	0.92±0.19	1.23±0.33 ^{d,f}	0.97±0.21	1.06±0.24 ^a
Premorbid antiplatelet drugs use	44 (21.5%)	23 (17.8%)	8 (27.6%)	8 (23.5%)	5 (38.5%)
Premorbid lipid-lowering drugs use	36 (17.6%)	16 (12.4%)	8 (27.6%) ^a	8 (23.5%)	4 (30.8%)

Values are mean ± SD, n (%) or median (interquartile range 25–75%).

E-ICA = Extracranial ICA; I-ICA = intracranial ICA; E-LAS = extracranial LAS; I-LAS = intracranial LAS; NGSP = National Glycohemoglobin Standardization Program.

¹Included patients with stenoses in both extracranial ICA and intracranial ICA/MCA. ^ap < 0.05, ^bp < 0.01, ^cp < 0.001, ^dp < 0.0001 vs. without LAS. ^ep < 0.05, ^fp < 0.001 vs. intracranial ICA/MCA.

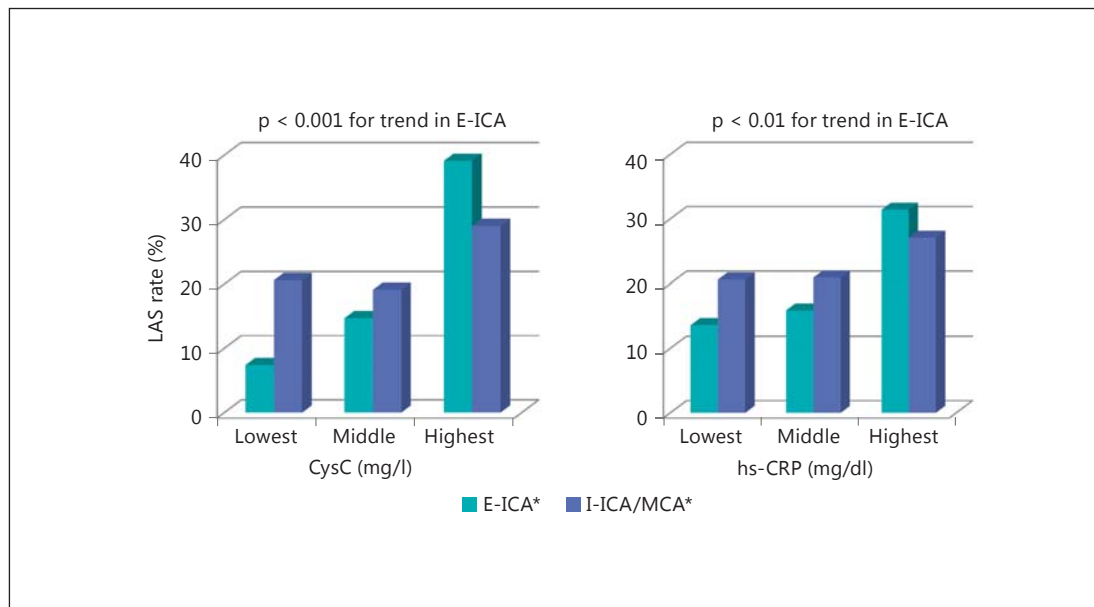


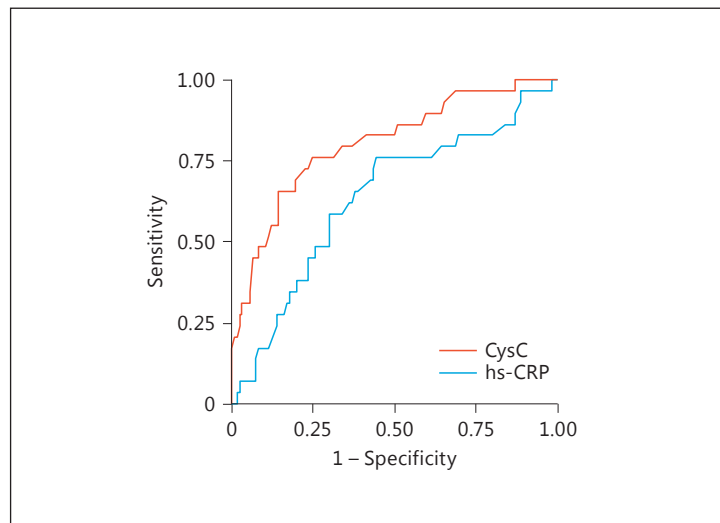
Fig. 2. Rates of extra- and intracranial LAS stratified by CysC and hs-CRP tertiles. There was a significant trend in the rate of extracranial ICA stenosis among the tertiles of CysC and hs-CRP (p for trend <0.001 and <0.01, respectively). CysC tertiles: lowest <0.84 mg/l, middle 0.84–1.04 mg/l, highest >1.04 mg/l. hs-CRP tertiles: lowest <0.69 mg/l, middle 0.69–1.80 mg/l, highest >1.80 mg/l. E-ICA = Extracranial ICA; I-ICA = intracranial ICA. * Included patients with stenosis in both extracranial ICA and intracranial ICA/MCA.

Table 3. ORs for LAS according to CysC levels

	Extracranial ICA		Intracranial ICA/MCA		Extra- + intracranial LAS	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Unadjusted model						
≤1.04 mg/l	1.0 (ref.)		1.0 (ref.)		1.0 (ref.)	
>1.04 mg/l	9.05 (3.63–22.56)	<0.0001	2.14 (0.95–0.78)	0.065	2.96 (0.92–9.49)	0.069
Per 1 SD	3.48 (2.07–5.84)	<0.0001	1.41 (0.89–2.24)	0.146	2.15 (1.12–4.14)	0.022
Adjusted model 1^a						
≤1.04 mg/l	1.0 (ref.)		1.0 (ref.)		1.0 (ref.)	
>1.04 mg/l	6.81 (2.63–17.63)	<0.0001	1.76 (0.76–4.09)	0.188	2.26 (0.67–7.62)	0.190
Per 1 SD	3.18 (1.87–5.43)	<0.0001	1.16 (0.69–1.95)	0.583	1.79 (0.87–3.69)	0.112
Adjusted model 2^b						
≤1.04 mg/l	1.0 (ref.)		1.0 (ref.)		1.0 (ref.)	
>1.04 mg/l	5.01 (1.51–16.63)	0.009	1.13 (0.41–3.15)	0.812	1.21 (0.18–8.06)	0.844
Per 1 SD	3.01 (1.58–5.72)	0.001	1.08 (0.58–2.03)	0.805	1.33 (0.43–4.06)	0.618

^a Model 1 was adjusted for age and sex. ^b Model 2 = model 1 plus additional adjustment for diabetes, CKD, current smoking, systolic blood pressure, HDL cholesterol, hs-CRP and premorbid lipid-lowering drugs use.

Fig. 3. Receiver operating characteristic curve analysis of CysC and hs-CRP cut-off point for the presence of extracranial ICA stenosis. Area under the curve (c-statistic) = 0.79 (CysC) and 0.63 (hs-CRP).



point, the sensitivity and specificity for extracranial ICA stenosis were 76 and 75%, respectively (c-statistic 0.79, 95% CI 0.69–0.89, $p < 0.001$). At a 1.60 mg/l hs-CRP cut-off point, the sensitivity and specificity for extracranial ICA stenosis were 75 and 54%, respectively (c-statistic 0.63, 95% CI 0.51–0.74, $p = 0.032$). CysC values improved the predictive ability for extracranial ICA stenosis as compared with hs-CRP values (fig. 3).

Discussion

In the present study, higher serum levels of CysC were associated with extracranial ICA stenosis, but not with intracranial ICA or MCA stenosis in patients with acute noncardioembolic ischemic stroke. To our knowledge, this is the first study to investigate an association

between kidney disease markers and extra- and intracranial large artery steno-occlusive disease. Our findings provide new insights into the brain and kidney interaction, which has been receiving much attention in recent years.

Kidney disease markers are important risk factors for cardiovascular events. A recent study using pooled analyses of prospective community-based cohorts demonstrated that albuminuria is associated with an increased risk of ischemic and hemorrhagic stroke and that decreased eGFR is only associated with an increased risk of ischemic stroke [19]. Moreover, microalbuminuria (UACR <30 mg/g creatinine) is thought to be a marker of vascular endothelial dysfunction and is associated with early neurological deterioration in patients with deep small infarcts [15] and cerebral small vessel disease, including cerebral white matter lesions and nonlobar cerebral microbleeds [20, 21]. In contrast, CysC is associated with chronic inflammation, which could be a promoting factor for atherosclerosis [13]. Thus, CysC may contribute to the process of carotid atherosclerosis via chronic inflammation. Cathepsin, a cysteine protease, contributes to plaque formation and rupture through its action on the vascular wall, which decomposes collagen and elastic fibers [22]. Therefore, it is possible that an imbalance between cysteine proteinase and CysC could impact changes in vascular structure and stenotic lesions. Additionally, an association between CKD and vascular medial calcification has been suggested [23]. In the present study, 3D CT angiography was only conducted on a small number of subjects, so there was no clear association between CysC and vascular calcification in the carotid artery. Further research is needed to determine whether CysC levels are associated with plaque characteristics of the carotid artery.

Differences in vascular structure may be an explanation for the discrepancy between kidney disease markers and extra- or intracranial LAS. More specifically, intracranial arteries are muscle arteries, and extracranial arteries are elastic arteries. Although the exact mechanism underlying large artery atherosclerosis remains unclear, intracranial arteries seem to be more susceptible to oxidative stress, which induces vascular endothelial dysfunction, while atherosclerosis or vascular calcification precedes its progression in extracranial arteries. Regarding factors associated with extra- or intracranial LAS, it has been reported that levels of hs-CRP were significantly higher in extracranial than in intracranial stenotic lesions [7]. However, as there is a possibility that various factors influence hs-CRP levels in the acute phase [9, 24], evaluation of hs-CRP in acute stroke can be tricky. In contrast, other research has suggested that CysC levels are less susceptible to the influence of the acute phase reaction [25]. Our findings suggest that CysC might be a more useful biomarker for identifying patients with advanced carotid artery disease as compared with hs-CRP. Furthermore, research on Caucasian populations without a history of stroke [1] revealed that male sex, hypertension, smoking and alcohol consumption were associated with extracranial atherosclerosis, while diabetes and insulin resistance were associated with intracranial atherosclerosis. In addition, an association between adiponectin levels and intracranial atherosclerosis has been reported in patients with acute ischemic stroke [4]. Given the results of these researches, several metabolic risk factors may promote intracranial atherosclerosis.

Several limitations of this study should be addressed. First, because it had a cross-sectional design, no clear causal relationship between CysC levels and extra- or intracranial LAS was found. Second, the present study was conducted on patients with acute ischemic stroke, and among of them, there were those who had been taking antiplatelet or lipid-lowering drugs before stroke onset; therefore, the influence of drug therapy could not be ruled out. Third, the degree of intracranial ICA and MCA stenosis was assessed using MRA, but not digital subtraction angiography, the gold standard, so the results could have been overestimated. Fourth, given the lack of statistical power stemming from the small number of patients with intracranial ICA stenosis, it was difficult to perform a meaningful analysis of intracranial LAS when it was classified into ICA and MCA. Fifth, in the setting of patients

without a history of stroke, a prospective study should be conducted to determine whether there are associations between kidney disease markers and extra- or intracranial LAS. Finally, although this preliminary study is limited by the small sample size, the results may provide some additional information linked to the mechanisms of carotid atherosclerosis.

In conclusion, higher CysC levels were strongly associated with symptomatic extracranial ICA stenosis in patients with noncardioembolic ischemic stroke. Determination of serum CysC may help in the identification of patients with extracranial carotid artery stenosis as well as renal impairment. However, a large-scale study is required to confirm our results. In addition, therapeutic interventions would be needed to determine whether suppressing the progression of renal impairment could reduce cerebrovascular events related to carotid atherosclerosis.

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Statement of Ethics

The Institutional Review Board of Chubu Rosai Hospital approved this study and waived the need for informed consent because this retrospective study was based on a review of medical records.

Disclosure Statement

The authors report no conflicts of interest.

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