Circulating Levels of High-sensitivity C-reactive protein are Associated with Intra-plaque Neovascularization and Intra-plaque Hemorrhage as Evaluated by Contrast-enhanced Ultrasonography and Magnetic Resonance Imaging

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Abstract

Background: Intra-plaque hemorrhage in carotid atheromatous plaque is a recognized high-risk state for ischemic events. Intra-plaque neovascularization can now be visualized by contrast-enhanced carotid ultrasonography (CEUS). These neovessels appear prone to rupture and may be a marker for high-risk of intra-plaque hemorrhage. This study aimed to investigate relationships among circulating biomarkers associated with carotid plaque instability and the presence of intra-plaque hemorrhage on magnetic resonance imaging (MRI) and intra-plaque neovascularization on CEUS in patients with advanced atherosclerotic plaques.

Methods: Fifty-one patients with mild to severe carotid stenosis were prospectively enrolled, and carotid ultrasonography, CEUS, and MRI were performed. Circulating levels of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1), pentraxin 3 (PTX3), myeloperoxidase (MPO), matrix metalloproteinase (MMP)-9, and high-sensitivity C-reactive protein (hs-CRP) were also measured.

Results: Carotid plaques were categorized into 3 groups based on MRI findings: fibrous tissue, n=13; lipid/necrotic core, n=30; and intra-plaque hemorrhage, n=8. Regarding plaque echogenicity, 24 plaques were hypoechoic, and 27 were non-hypoechoic. Intra-plaque neovascularization on CEUS was observed in 14 plaques. Multivariate logistic regression analysis identified hypoechoic plaque (odds ratio, 63.126; 95% confidence interval, 1.102–3616.91) and serum hs-CRP (1.064; 1.002–1.130) as factors significantly associated with intra-plaque hemorrhage. Hypoechoic plaque (30.362; 2.462–374.37), and serum hs-CRP (1.066; 1.003–1.132) were revealed as factors significantly associated with intra-plaque neovascularization.

Conclusions: Hypoechoic plaque and hs-CRP levels were identified as common predictors of intra-plaque hemorrhage and intra-plaque neovascularization. Longitudinal studies are warranted to clarify the roles of intra-plaque neovascularization on CEUS and hs-CRP levels in intra-plaque hemorrhage and ischemic stroke.

Key words

unstable plaque, carotid ultrasound, black-blood, biomarker

Subject Terms

Biomarker, Magnetic Resonance Imaging, Ultrasound, Cerebrovascular Disease, Stroke

Introduction

The presence of atherosclerotic plaques in the carotid artery is an important risk factor for cerebral

infarction¹⁾. Evaluation of carotid plaque vulnerability has been performed by carotid ultrasonography or magnetic resonance imaging (MRI). Hypoechoic plaque on carotid ultrasound and a lipid core and/or in-

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tra-plaque hemorrhage on MRI are features that are well-known to be highly associated with vulnerable plaques²⁻⁴). In particular, the presence of intra-plaque hemorrhage on MRI is currently recognized as a high-risk state for ischemic events resulting from plaque destabilization⁵⁻⁷). Several histopathological studies demonstrated that intra-plaque neovascularization is observed in advanced atherosclerotic lesions and these neovessels could be prone to rupture^{8,9}). The presence of intra-plaque neovascularization can now be evaluated by contrast-enhanced ultrasonography (CEUS), which may be useful for the characterization of both vulnerable carotid plaque features¹⁰ and luminal stenosis. However, whether the presence of intra-plaque neovascularization is a biomarker directly predictive of intra-plaque hemorrhage remains uncertain. In addition to imaging biomarkers, various circulating biomarkers such as high-sensitivity C-reactive protein (hs-CRP)^{11,12}, pentraxin 3 (PTX-3)¹³, and soluble lectin-like oxidized low-density lipoprotein receptor 1 (sLOX-1)¹⁴ are known to be associated with vascular inflammation and plaque instability. Matrix metalloproteinase 9 (MMP-9)^{15,16} and myeloperoxidase (MPO)¹⁷ are known to be associated with plaque destabilization, rupture, or erosion. No information has been published regarding the difference in risk profiles including these circulating biomarkers between intra-plaque hemorrhage and intra-plaque neovascularization. Therefore, the aim of the present study was to investigate relationships between the above circulating biomarkers and the presence of intra-plaque hemorrhage on MRI and intra-plaque neovascularization on CEUS in patients with advanced atherosclerotic plaques.

Patients and Methods

Study population

Fifty-one patients (43 men, 8 women; mean age, 73.6 ± 9.34 years) with mild to severe carotid stenosis on carotid ultrasonography (≥50% in cross-sectional area) diagnosed from April 2013 to May 2015 were prospectively enrolled in this study. Based on clinical data including neuroimaging data, the ischemic stroke subtype was diagnosed on admission according to the Trial of ORG 10172 in Acute Stroke Treatment classification as: 1) large-artery atherosclerosis, 2) cardioembolism, 3) small-vessel occlusion, 4) stroke of other determined etiology, and 5) stroke of undetermined etiology¹⁸). The exclusion criteria were 1) serious concurrent disease, 2) ischemic stroke subtype of other determined etiology and undetermined

etiology, 3) severe inflammatory disease or autoimmune disease (white blood cells >1.0 × 10⁴ mm³, CRP >3.0 mg/dl), and 4) use of steroids or non-steroidal anti-inflammatory drugs. This study was conducted in a single hospital, and the study protocol was approved by the St. Marianna University Bioethics Committee (no. 2419). Informed consent to participate was obtained from all patients.

Clinical data collection

Background factors and laboratory test values were determined for each patient at baseline. Age, sex, presence of hypertension, diabetes mellitus, dyslipidemia, smoking habit (Brinkman index), and history of any cardiovascular events were recorded. Definitions of risk factors were as follows. Hypertension was defined as ≥2 blood pressure measurements of ≥140/90 mmHg or previous diagnosis of hypertension and use of antihypertensive agents. Diabetes was considered to be present if the patient showed an HbA1c ≥6.1% or if treatment for diabetes was being administered. Abnormal lipid metabolism was defined as total cholesterol ≥220 mg/dL, neutral fat levels ≥150 mg/dL, HDL cholesterol <40 mg/dL, or use of oral statin agents.

MRI imaging

Black-blood (BB) carotid artery MRI was performed on a 1.5-T scanner (Achieva Nova Dual; Philips Healthcare, Best, the Netherlands) using a 16-channel NV coil. For positioning of greatest wall thickness, a standard imaging protocol was used, i.e., time-of-flight (TOF), T1-weighted, and T2-weighted. For short-axis T1-weighted images (2-dimensional turbo spin-echo), the parameters were recovery time (TR) 1 cardiac cycle (1 R-R interval), echo time (TE) 15 ms, matrix 153 × 192, thickness 3 mm, field of view (FOV) 150 mm, number of samples (NSA) 6, and fat saturation (+). For short-axis T2-weighted images (2D-TSE), parameters were TR 2 cardiac cycles (2 R-R intervals), TE 80 ms, matrix 153 × 192, thickness 3 mm, FOV 150 mm, NSA 8, and fat saturation (+). For TOF 2D-TSE, parameters were TR 23 ms, TE 6.9 ms, matrix 120 × 240, thickness 1.25 mm, number of excitations 1, and fat saturation (+).

TOF, T1-weighted, and T2-weighted images were used for the characterization of carotid plaques. The signal intensity ratio relative to the ipsilateral sternocleidomastoid muscle (plaque/muscle ratio) was calculated, and high intensity was defined as a plaque/muscle ratio >2¹⁹). Plaque characteristics were

classified into 3 categories: 1) fibrous tissue, 2) lipid/necrotic core, and 3) intra-plaque hemorrhage⁴⁾ (**Figure 1**). To test interobserver reliability, the intraclass correlation coefficient (ICC) was calculated for three assessors (ICC=0.791, 0.360–0.944).

Ultrasonography

Carotid US with a 7.5-MHz linear probe (SSA-770A Aplio or SSA-660A Xario; Toshiba Medical, Tokyo, Japan, and HDI-3000; Hitachi, Tokyo, Japan) was performed within 3 months before or after the BB-MRI examination. B-mode imaging, color and power Doppler imaging, and CEUS were performed using Sonazoid (perfluorobutane microbubbles; Daiichi Sankyo Pharmaceutical, Tokyo, Japan). Maximum percent area stenosis (%AS) was calculated from measurements on serial cross-sectional images by B-mode ultrasonography using the following formula:

$$\%AS = [1 - (\text{area of residual lumen}/\text{area of normal vessel})] \times 100.$$

If the %AS was greater than 50%, the patient was enrolled in this study, and peak systolic flow velocity at the stenotic lesion was recorded. Intima-media thickness (IMT) was measured in the posterior wall of each carotid artery as the distance between the leading edge of the first and second echogenic lines. Maximum IMT (maxIMT) was determined as the larger IMT of the two carotid arteries (common and internal carotid arteries). Carotid plaques were classified into two groups: hypoechoic plaque or non-hypoechoic plaque.

A total of 16 μL of Sonazoid was dissolved in 2 mL of water, and an intravenous bolus injection of the contrast agent suspension was injected into the antecubital vein for a dose of 0.15 mL/kg. The acoustic power for harmonic US was set with a mechanical index of 0.2–0.3, depth 3 cm, and focus 2.5 cm. Car-

Figure 1. Characterization of carotid plaque by black-blood magnetic resonance imaging and contrast-enhanced ultrasonography (CEUS) imaging
A. Angiogram of the left internal carotid artery (ICA) showing high-grade stenosis.
B. Color-coded duplex ultrasound of the ICA with hypoechoic atherosclerotic plaque.
C. The plaque shows high signal intensity on time-of-flight magnetic resonance angiography (C-1), and T1-weighted imaging (C-2).
D. Sonazoid (CEUS) showing punctate contrast enhancement in the plaque (▼)

otid plaque was evaluated from the longitudinal and transverse views for about 5 min after contrast injection. Presence of an enhanced lesion was identified based on echogenic reflectors of microbubbles in the plaque (**Figure 1**). The kappa coefficient for diagnostic concordance of hypoechoic plaque between two examiners was 0.750.

Measurements of biomarkers

All blood samples were centrifuged at 3000 rpm for 10 min at 4°C. Serum and plasma were separated and stored at -80°C until analysis. Levels of hs-CRP, MMP-9, PTX-3, and MPO were measured in duplicate using commercially available ELISA kits: the CardioPhase High-sensitivity C-Reactive Protein system (Siemens Medical Solutions, Erlangen, Germany), the human MMP-9 ELISA System (GE Healthcare, Buckinghamshire, UK), the Human Pentraxin3 ELISA System, (Perseus Proteomics, Tokyo, Japan), and the Human MPO ELISA System (Merckodia AB, Uppsala, Sweden). Serum sLOX-1 levels were measured by an enzyme immunoassay, as previously described²⁰, except that immobilized mouse monoclonal anti-sLOX-1 and 96-well EIA plates (Corning, NY) were used. Serum levels of LOX-1 ligand containing apoB (LAB) were measured by an enzyme immunoassay with immobilized recombinant LOX-1, as previously described²⁰, except that a chimeric standard protein²¹ and 96-well EIA plates (Greiner, Kremsmuenster, Austria) were used.

Statistical analyses

Characteristics of the patients are presented as means and standard deviation unless otherwise indicated. Multivariate logistic regression analysis was performed to determine which baseline characteristics were independently associated with intra-plaque hemorrhage on MRI and intra-plaque neovascularization on CEUS. Variables were included in this model if univariate *p*-values were less than 0.1. A two-sided *p*-value less than 0.05 was considered significant. All statistical analyses were performed using SPSS version 22 (IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY).

Results

Patient demographics and clinical characteristics including carotid US findings, BB-MRI plaque findings, and values of circulating biomarkers are shown in **Table 1**. MRI diagnoses of plaque characteristics were as follows: fibrous tissue, n=13; lipid/necrotic

core, n=30; and intra-plaque hemorrhage, n=8. The odds ratios (ORs) and 95% confidence intervals (95% CIs) of each parameter for predicting intra-plaque hemorrhage by BB-MRI and intra-plaque neovascularization by CEUS are shown in **Table 2**. **Figures 2** and **3** show plots of the respective biomarkers in the presence or absence of intra-plaque hemorrhage and intra-plaque neovascularization. Although the difference was not statistically significant, the serum hs-CRP level tended to be high in intra-plaque hemorrhage and intra-plaque neovascularization. In intra-plaque hemorrhage, univariate logistic regression analyses revealed three factors with *p*-values less than 0.1: brain hemorrhage, hypoechoic plaque, and serum hs-CRP. Multivariate logistic regression analysis showed that hypoechoic plaque (OR 63.126, 95% CI 1.102–3616.91) and serum hs-CRP (OR 1.064, 95% CI 1.002–1.130) were significantly associated with intra-plaque hemorrhage (**Table 3**). Similarly, multivariate logistic regression analysis of factors associated with intra-plaque neovascularization identified hypoechoic plaque on carotid US (OR 30.362, 95% CI 2.462–374.37) and serum hs-CRP (OR 1.066, 95% CI 1.003–1.132) as significant factors (**Table 4**). No other biomarkers were predictive of imaging characteristics of plaque instability in MRI-BB and CEUS.

Discussion

Previously described pathological features of vulnerable plaques have included a thin fibrous cap with a large lipid core, intra-plaque hemorrhage, specific luminal surface morphology such as irregular and ulcerated areas, and active intra-plaque inflammation²². These pathological features can be evaluated by several imaging modalities, such as positron emission tomography²³, computed tomographic angiography, MRI²⁴, and CEUS²⁵. In this study, intra-plaque hemorrhage was detected by MRI plaque imaging. CEUS has recently been used to visualize intra-plaque neovascularization as an element of plaque vulnerability¹⁰.

As a plaque forms and enlarges and luminal stenosis progresses, regional blood flow velocity and shear stress increase. High shear stress is associated with a large lipid core and plaque instability²³. Plaque rupture occurs at sites with excessive shear stress^{27,28}, and areas where shear stress is concentrated tend to become future plaque rupture points²⁹. Shear stress has been identified as a trigger for the development of arteriogenesis and neovasculariza-

Table 1. Demographic and Clinical Characteristics of Study Patients

Characteristic	Patients (n=51)
Male, n (%)	43 (84.3)
Age, years	73.5±9.4
Comorbidity, n (%)	
Hypertension	44 (86.3)
Dyslipidemia	27 (52.9)
Diabetes mellitus	17 (33.3)
Smoking (Brinkman index)	760.4±665.5
Cerebrovascular disease, n (%)	
TIA	4 (7.8)
Brain infarction	20 (39.2)
Brain hemorrhage	4 (7.8)
Coronary artery disease, n (%)	11 (21.6)
Peripheral artery disease, n (%)	8 (15.7)
Chronic kidney disease, n (%)	14 (27.5)
Systolic blood pressure, mmHg	142.2±26.0
Diastolic blood pressure, mmHg	75.5±14.4
Total cholesterol, mg/dL	176.3±39.4
Triglyceride, mg/dL	115.5±66.0
HDL cholesterol, mg/dL	49.5±15.5
LDL cholesterol, mg/dL	104.9±32.0
Blood sugar, mg/dL	133.4±46.1
HbA1c, %	6.2±0.9
Current medications, n (%)	
Statin	24 (47.1)
Carotid ultrasound findings	
Area ratio (%)	75.6±12.2
Peak systolic flow (cm/s)	164.4±121.9
Max IMT (mm)	4.3±1.2
Morphology of plaque, n (%)	
Heterogeneous	20 (39.2)
Hypochoic	24 (47.1)
BB-MRI plaque findings, n (%)	
Fibrous tissue	13 (25.5)
Lipid/necrotic core	30 (58.8)
Intra-plaque hemorrhage	8 (15.7)
Biomarker	
LAB (mg cs/L)	5.9±3.0
sLOX-1 (ng/L)	1402.4±952.8
MPO (µg/L)	91.4±65.8
PTX3 (ng/mL)	4.1±4.0
MMP-9 (ng/mL)	10.2±5.1
Hs-CRP (ng/mL)	7775.5±16624.6

TIA, transient ischemic attack; HDL, high-density lipoprotein; LDL, low-density lipoprotein; max IMT, maximum value of intima media thickness; BB-MRI, black-blood MR imaging; LAB, LOX-1 ligand containing apoB; sLOX, soluble form of lectin-like oxidized LDL receptor 1; MPO, myeloperoxidase; PTX3, pentraxin3; MMP-9, matrix metalloproteinase-9; hs-CRP, high-sensitivity C-reactive protein.

tion³⁰). However, our study demonstrated that the hypochoic nature of the plaque and hs-CRP levels were also factors commonly associated with intra-plaque hemorrhage and intra-plaque neovascularization. These results are reasonable because inflammation of carotid plaque may play an important role in

developing plaque instability that is independent of the severity of stenosis.

Several promising biomarkers predictive of plaque instability have been found. Hayashida et al. reported that serum sLOX-1 levels were significantly higher in patients with acute coronary syndrome

Table 2. Univariate Odds Ratio of Confounders for Intra-plaque Hemorrhage on Black-blood MRI and Intra-plaque Neovascularization on Contrast-enhanced Carotid Ultrasound

	Intra-plaque hemorrhage (+)				Intra-plaque neovascularization (+)			
	Univariate analysis		Univariate analysis		Univariate analysis		Univariate analysis	
	OR	95%CI	p		OR	95%CI	p	
Age	.977	.907	1.054	.551	.964	.903	1.029	.267
Male sex	-	.000	-	.999	-	.000	-	.999
Comorbidity								
Hypertension	.395	.062	2.516	.325	.444	.086	2.303	.334
Dyslipidemia	.870	.192	3.936	.856	.850	.248	2.911	.796
Diabetes mellitus	.000	.000	-	.998	.738	.193	2.825	.658
Smoking (Brinkman index)	1.000	.999	1.002	.353	1.000	.999	1.001	.449
Cerebrovascular disease								
TIA	.000	.000	-	.999	.872	.083	9.157	.909
Brain infarction	3.111	.652	14.845	.155	.525	.139	1.984	.342
Brain hemorrhage	6.833	.805	58.022	.078	2.917	.369	23.038	.310
Coronary artery disease	.471	.052	4.304	.505	.519	.097	2.768	.442
Peripheral artery disease	.735	.078	6.945	.788	.000	.000	-	.999
Chronic kidney disease	.330	.037	2.958	.322	.645	.150	2.771	.555
Systolic blood pressure (mmHg)	1.004	.975	1.034	.792	.997	.974	1.022	.831
Diastolic blood pressure (mmHg)	1.027	.976	1.081	.301	1.022	.980	1.067	.309
Total cholesterol (mg/dl)	1.002	.983	1.022	.833	.994	.979	1.010	.487
Triglyceride (mg/dl)	1.004	.993	1.014	.496	1.000	.991	1.010	.959
HDL cholesterol (mg/dl)	.986	.933	1.041	.599	.994	.953	1.035	.757
LDL cholesterol (mg/dl)	.995	.971	1.019	.675	.992	.973	1.012	.424
Blood sugar (mg/dl)	1.000	.983	1.017	.991	1.002	.990	1.015	.721
HbA1c	.396	.088	1.782	.227	.958	.497	1.847	.898
Current medications								
Statin	4.167	.735	23.064	.102	1.176	.343	4.029	.796
Carotid ultrasound findings								
Area ratio	.992	.932	1.055	.796	1.058	.997	1.124	.064
Peak systolic flow (cm/sec)	1.004	.998	1.009	.212	1.010	1.004	1.017	.001
Max IMT	.846	.442	1.618	.613	1.460	.882	2.417	.141
Plaque morphology								
Heterogeneous	0.918	.194	4.351	.914	.525	.139	1.984	.342
Hypochoic plaque	10.706	1.207	94.959	.033	12.500	2.406	64.932	.003
Intra-plaque neovascularization	3.30	.696	15.642	.133	-	-	-	-
BB-MRI plaque findings								
Fibrous tissue	0.000	0.000	0.000	0.999	1.244	.313	4.954	.756
Lipid/necrotic core	0.000	0.000	0.000	0.998	.406	.116	1.426	.160
Intra-plaque hemorrhage	-	-	-	-	.330	.696	15.642	.133
Biomarkers								
Serum LAB(mg cs/L)	0.925	0.703	1.217	.579	.796	.612	1.037	.091
Serum LOX-1 (ng/L)	1.000	.999	1.001	.591	1.000	.999	1.001	.950
Plasma MPO (μg/L)	1.001	.990	1.012	.833	1.006	.997	1.014	.221
Plasma PTX3 (ng/mL)	1.004	.833	1.209	.970	.895	.696	1.150	.385
Serum MMP-9 (ng/mL)	1.063	.923	1.224	.398	.991	.877	1.120	.886
Serum hs-CRP (mg/mL)	1.034	0.995	1.075	.091	1.035	0.994	1.077	.092

TIA, transient ischemic attack; US, ultrasound; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IMT,

maximum-intima-media thickness; BB-MRI, black-blood magnetic resonance imaging; LAB, LOX-1 ligand containing apoB; sLOX-1, soluble form of lectin-like oxidized LDL receptor 1; MPO, myeloperoxidase; PTX3, pentraxin 3; MMP-9, matrix metalloproteinase 9; hs-CRP, high-sensitivity C-reactive protein.

(ACS) than in patients without ACS, and the sensitivity and specificity of sLOX-1 >1.0 ng/mL for the diagnosis of ACS were reported to be 81% and 75%, respectively¹⁴). MMP-9 is a zinc-dependent endopeptidase that degrades the extracellular matrix, and this enzyme is elevated in culprit lesions. Alvarez et al.³¹) demonstrated a significant increase of serum MMP-9

concentrations in patients with symptomatic carotid artery stenosis and suggested MMP-9 as a biomarker of carotid plaque instability. Brennan et al.¹⁷) assessed the value of plasma levels of MPO in 604 consecutive patients with chest pain and reported that a single initial measurement of plasma MPO independently predicted early risk for myocardial infarction. Plasma

Figure 2. Box and whiskers plots of biomarker levels in intra-plaque hemorrhage

Box plots showing the distribution of biomarker levels by according to the presence or absence of intra-plaque hemorrhage. IPH, intra-plaque hemorrhage; LAB, LOX-1 ligand containing apoB; sLOX-1, soluble form of lectin-like oxidized LDL receptor 1; MPO, myeloperoxidase; PTX3, pentraxin 3; MMP-9, matrix metalloproteinase 9; hs-CRP, high-sensitivity C-reactive protein.

levels of PTX3 are associated with the presence of atherosclerotic plaques and prevalent vascular disease¹³⁾³²⁾, and abundant localization of PTX-3 in areas of intra-plaque hemorrhage was demonstrated by immunohistochemistry³³⁾. Of these promising biomarkers, only hs-CRP was found to be a biomarker predictive of intra-plaque hemorrhage and intra-plaque neovascularization by multivariate logistic regression analysis in the present study.

The source of neovascularization is direct branching from the vasa vasorum or intimal surface. These areas are very fragile, and plaque hemorrhage can easily occur³⁴⁾³⁵⁾. Evaluation of neovascularization is therefore important in assessing plaque instability. Currently, to the best of our knowledge, only a few studies have assessed the association between carotid artery plaque neovascularization and circulating biomarker levels. Serum levels of CD146³⁶⁾ and vascular endothelial growth factor³⁷⁾ have recently been reported as biomarkers associated with histologically determined neovascularization. Jaipersad et al.³⁸⁾ demonstrated correlations between the levels of

CD14+/CD16-/CCR2+ monocytes and intra-plaque neovascularization on CEUS. Further studies are necessary to identify the best biomarker to predict intra-plaque neovascularization and intra-plaque hemorrhage.

It is reasonable to assume that microvessels formed in plaque are prone to rupture, but no significant association between intra-plaque neovascularization and intra-plaque hemorrhage was observed in the present cross-sectional study. Hypoechoic plaques seen on carotid US are pathologically associated with large lipid cores and intra-plaque hemorrhage and are widely recognized as plaques that confer a high risk of cerebral infarction²⁾³⁾³⁹⁾. However, distinguishing between lipid cores and intra-plaque hemorrhage is often difficult on the basis of hypoechoic findings alone. Further longitudinal follow-up studies are needed to verify whether the evaluation of echogenicity, peak systolic flow velocity, intra-plaque neovascularization, and hs-CRP levels could be used to identify carotid plaque instability.

This study has several limitations. First, the con-

Figure 3. Box and whiskers plots of biomarker levels in intra-plaque neovascularization

Box plot showing the distribution of biomarker levels according to the presence or absence of intra-plaque neovascularization. IPN, intra-plaque neovascularization; LAB, LOX-1 ligand containing apoB; sLOX-1, soluble form of lectin-like oxidized LDL receptor 1; MPO, myeloperoxidase; PTX3, pentraxin 3; MMP-9, matrix metalloproteinase 9; hs-CRP, high-sensitivity C-reactive protein.

Table 3. Multivariate Logistic Regression Analysis for Intra-plaque Hemorrhage on Black-blood Magnetic Resonance Imaging

	Multivariate analysis		
	OR	95%CI	p
Brain hemorrhage	10.768	0.387 299.38	0.161
Hypochoic plaque	63.126	1.102 3616.91	0.045
Serum hs-CRP (mg/mL)	1.064	1.002 1.130	0.041

hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval.

trast effect with Sonazoid is caused by presence in the plaque of echogenic reflectors of microbubbles; therefore, the more hypoechoic a plaque, the easier it may be to assess these contrast effects, and this may have influenced our results. However, hyperechoic plaques are widely known to be associated with a lower risk of vascular events than hypoechoic plaques, so this does not negate the value of the CEUS findings. Echogenicity in this study was initially evaluated qualitatively by an examiner without using a quantitative technique such as a gray-scale median⁴⁰⁾

or integrated backscatter⁴¹⁾. However, screening studies for unstable plaques are also usually qualitative. In a longitudinal study to investigate changes over time in plaque brightness, a quantitative method would be required. Second, imaging to evaluate fibrous cap thinning or rupture was not performed. Intra-plaque hemorrhage is followed by plaque rupture in the process leading to cerebral infarction, and fibrous cap rupture is an essential feature. The addition of MRI to evaluate the fibrous cap is clinically important and would be highly valuable in future studies.

Table 4. Multivariate Logistic Regression Analysis for Intra-plaque Neovascularization on Contrast-enhanced Carotid Ultrasound

	Multivariate analysis			
	OR	95%CI		p
Area ratio	1.011	0.916	1.116	0.828
Peak systolic flow (cm/sec)	5.644	0.608	52.38	0.128
Hypoechoic plaque	30.362	2.462	374.37	0.008
Serum LAB (mg cs/L)	0.975	0.695	1.370	0.886
Serum hs-CRP (ng/mL)	1.066	1.003	1.132	0.040

LAB, LOX-1 ligand containing apoB; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval.

Third, this study used MRI to evaluate intra-plaque hemorrhage and the lipid core, but pathological studies to confirm these findings were not performed in any patient. Future studies should include correlation with pathological findings.

Conclusion

In conclusion, hypoechoic plaque and hs-CRP levels were found to be factors commonly predictive of intra-plaque hemorrhage and intra-plaque neovascularization. Further longitudinal studies are necessary to clarify the roles of intra-plaque neovascularization and hs-CRP levels in intra-plaque hemorrhage.

Disclosures

Kagehiro Uchida is an employee of Biomarker Science. The other authors report no conflicts.

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