

Utility of Complementary Magnetic Resonance Plaque Imaging and Contrast-Enhanced Ultrasound to Detect Carotid Vulnerable Plaques

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Background—We aimed to improve the assessment quality of plaque vulnerability with combined use of magnetic resonance imaging and contrast-enhanced ultrasound (CEUS).

Methods and Results—We prospectively enrolled 71 patients with internal carotid artery stenosis who underwent carotid endarterectomy and performed preoperative CEUS and magnetic resonance plaque imaging. We distinguished high-signal-intensity plaques (HIPs) and non-HIPs based on magnetization-prepared rapid acquisition with gradient echo images. We graded them according to the CEUS contrast effect and compared the CEUS images with the carotid endarterectomy specimens. Among the 70 plaques, except 1 carotid endarterectomy tissue sample failure, 59 were classified as HIPs (43 symptomatic) and 11 were classified as non-HIPs (5 symptomatic). Although the magnetization-prepared rapid acquisition with gradient echo findings alone had no significant correlation with symptoms ($P=0.07$), concomitant use of magnetization-prepared rapid acquisition with gradient echo and CEUS findings did show a significant correlation ($P<0.0001$). CEUS showed that all 5 symptomatic non-HIPs had a high-contrast effect. These 5 plaques were histopathologically confirmed as vulnerable, with extensive neovascularization but only a small amount of intraplaque hemorrhage.

Conclusions—Complementary use of magnetic resonance imaging and CEUS to detect intraplaque hemorrhage and neovascularization in plaques can be useful for evaluating plaque vulnerability, consistent with the destabilization process associated with neovessel formation and subsequent intraplaque hemorrhage. (*J Am Heart Assoc.* 2019;8:e011302. DOI: 10.1161/JAHA.118.011302.)

Key Words: carotid artery plaque • carotid magnetic resonance imaging • carotid ultrasound • cerebral infarction • contrast-enhanced ultrasound

Carotid stenosis with atherosclerotic plaque formation is a well-known risk factor for artery-to-artery embolism.

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Qualitative evaluations of plaques, to determine the stroke risk, in addition to quantitative assessment of factors, such as the severity of stenosis, are being increasingly performed. Vulnerable plaques are histopathologically characterized by the presence of a lipid necrotic core, intraplaque hemorrhage (IPH), intraplaque neovascularization (IPN), active inflammation, and a thin/ruptured fibrous cap. Magnetic resonance imaging (MRI) of plaques is widely performed, including magnetization-prepared rapid acquisition with gradient echo (MPRAGE). High-signal-intensity plaques (HIPs), detected by MPRAGE, indicate necrotic cores with IPH.¹ However, we sometimes encounter symptomatic patients without HIPs on MPRAGE. Recent studies have shown the efficacy of evaluating IPN using contrast-enhanced ultrasound (CEUS).² Although these 2 modalities evaluate different components of plaque vulnerability, in view of the process of atherosclerosis progression, their complementary use may improve the diagnostic quality. We, therefore, assessed the diagnostic accuracy of plaque vulnerability using both CEUS and MPRAGE.

Clinical Perspective

What Is New?

- We showed the complementary use of magnetic resonance imaging and contrast-enhanced ultrasound to detect intraplaque hemorrhage and neovascularization, which improves the diagnostic quality of the assessment of plaque vulnerability and which was confirmed histopathologically.

What Are the Clinical Implications?

- Although magnetization-prepared rapid acquisition with gradient echo is useful for detecting intraplaque hemorrhage, during the early stage of destabilization before the collapse of neovessels, we may fail to detect vulnerability using only magnetization-prepared rapid acquisition with gradient echo.
- Contrast-enhanced ultrasound may be more available to evaluate plaque vulnerability for screening, when considering cost and facilities, compared with magnetic resonance imaging, especially in outpatient clinics.
- Contrast-enhanced ultrasound also allows easy follow-up valuation during the early stage of destabilization before the collapse of neovessels, resulting in intraplaque hemorrhage, which is reasonable in view of the process of atherosclerosis progression.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Patient Enrollment

We prospectively enrolled consecutive patients with internal carotid artery stenosis who underwent carotid endarterectomy (CEA) and preoperatively examined CEUS and MPRAGE images of carotid arteries at the National Cerebral and Cardiovascular Center (Osaka, Japan) from July 2010 to June 2014. The Ethics Committee of the National Cerebral and Cardiovascular Center approved this study. Written informed consent was obtained from all patients before enrollment. The exclusion criteria were a previous allergic reaction to perflubutane (perfluorobutane) (Sonazoid) or eggs because the lipid-stabilized suspension of perflubutane contains egg yolk.

Patient Characteristics

Data collected included vascular risks, stenosis severity, and symptoms associated with previous ischemic events on the ipsilateral side. Symptomatic events were classified as a transient ischemic attack (TIA), amaurosis fugax, or cerebral infarction. TIA was defined as a sudden, focal neurological

deficit that lasted <24 hours. Amaurosis fugax was defined as a sudden, temporary loss of vision in the ipsilateral eye. Cerebral infarction was defined as a sudden, focal neurological deficit that lasted ≥ 24 hours. Vascular risk factors were defined as follows. Hypertension was defined as a blood pressure of $\geq 140/90$ mmHg and/or antihypertensive drug use. Diabetes mellitus was confirmed according to established guidelines and/or use of medication for diabetes mellitus. Dyslipidemia was defined as a low-density lipoprotein level of >3.6 mmol/L, a high-density lipoprotein level of <1.0 mmol/L, a triglyceride level of >3.8 mmol/L, and/or statin use. Stenosis severity was assessed according to the NASCET (North American Symptomatic Carotid Endarterectomy Trial),³ with computed tomographic angiography or MR angiography. Symptomatic plaques were defined as those associated with a history of TIA, cerebral infarction, or both on the ipsilateral side.

CEUS Image Analysis

Carotid ultrasound examination was performed using an LOGIQ E9 ultrasound system (GE Yokogawa Medical Systems, Hino, Japan) with a linear probe (6–9 MHz phased-array transducer).

CEUS examinations were performed using the phase-inversion mode to delineate neovessels. The mechanical index was 0.2 to 0.3. Image depth was adjusted to 4 to 5 cm, and the focus position was 3 to 4 cm.

Sonazoid,⁴ a lipid-stabilized suspension of perflubutane gas microbubbles (0.01 mL/kg body weight), was injected as an IV bolus, followed by a 10-mL saline flush through an antecubital vein. It was necessary to discriminate the true contrast effect from artifacts, which appeared as bright echoes. We initiated observation before the injection of contrast agent and traced the microbubbles moving into the plaques from the vessel lumen or adventitial side to eliminate artifacts. The appearance of microbubbles was observed within 10 to 20 seconds after injection, and we observed the plaques and recorded images as cine clips in the short and long axes.

Intraplaque neovessels were identified by the movement of the echogenic reflectors of microbubbles within the plaque. Neovessels of the plaques were delineated by accumulation of these cine memory images in the phase-inversion mode. Evaluable images were acquired for at least 5 minutes after injecting each bolus. The contrast effects were classified semiquantitatively on a scale of grade 0 to 3, where 0 indicates absent; 1, small; 2, large; and 3, extensive. Plaques with no visible microbubbles were defined as grade 0; plaques with a small number of microbubbles were defined as grade 1; plaques with many microbubbles seen constantly were defined as grade 3; and plaques with microbubbles between grades 1 and 3 were defined as grade 2. These 4 grades were further categorized into 2 groups: low-contrast effect group

(grade 0–1); high-contrast effect group (grade 2–3) (Video S1). Two observers (R.M. and K.S.) independently graded the cine clips offline at different time points, with no prior knowledge of the patients' clinical information. Disagreements were resolved through consensus (κ statistic, 0.75).

MR Imaging

Images were obtained with a MAGNETOM Sonata 1.5-T or a Verio 3-T MR scanner (Siemens, Munich, Germany), with standard neck and spine coils. Plaque imaging was performed using MPRAGE, including the external vessel wall and vascular lumen, transaxially with the null blood condition (effective inversion time, 660 milliseconds; repetition times, 1500 milliseconds [Sonata] and 1400 milliseconds [Verio]) and the water excitation technique to suppress fat signals. Other imaging variables were as follows: echo times, 5.0 milliseconds (Sonata) and 2.68 milliseconds (Verio); field of view, 180×180 mm (Sonata) and 250×250 mm (Verio); matrix, 256×204 (Sonata) and 256×256 (Verio); slice thickness, 1.25 mm (Sonata) and 1.5 mm (Verio); 56 partitions covering 70 mm around the carotid bifurcation; and data acquisition time, 5 minutes (Sonata) and 3 minutes 46 seconds (Verio). Multislab 3-dimensional time-of-flight MR angiography was also performed to determine the lumen shape and plaque morphological features with the following parameters: echo times, 4.4 milliseconds (Sonata) and 3.69 milliseconds (Verio); repetition times, 35 milliseconds (Sonata) and 25 milliseconds (Verio); and the same spatial resolution as for MPRAGE.^{5,6}

We calculated the signal intensity ratio of the carotid plaque component by dividing the plaque signal intensity by the muscle signal intensity. Plaques with a signal intensity ratio of >2 were categorized as having high-signal intensity. A single, blinded investigator (R.M.) obtained all measurements. The reproducibility of the signal intensity ratio with MRI was assessed in all plaques using measurements performed by a blinded second reader. Disagreements were resolved through consensus (κ statistic, 0.95). We defined plaques with high-signal intensity as HIPs and those without high-signal intensity as non-HIPs.

Only 2 plaques (2.8%) evaluated using 1.5-T MRI were included in this study, both of which were diagnosed as HIPs without interobserver disagreement. When the signal intensity ratio is different between 1.5-T and 3-T MRI, the plaques may tend to show higher signal in cases using 3-T MRI than in those using 1.5-T MRI. We considered that inclusion of the 2 plaques in the HIP group could not affect the results.

Histological Analysis

For the histological analysis, CEA specimens were immersed immediately in fixative solution (Histochoice; Amresco, Solon,

Table. Characteristics of Patients

Characteristics	Symptomatic Patients (n=48)	Asymptomatic Patients (n=22)	P Value	
Age, y	71.1±6.6	72.3±6.3	0.46	
Men	47 (98)	21 (96)	0.53	
Risk factors				
Current smoker	10 (21)	2 (9)	0.32	
Diabetes mellitus	11 (23)	10 (46)	0.056	
Hypertension	40 (83)	20 (91)	0.49	
Dyslipidemia	33 (69)	19 (86)	0.15	
Plaque characteristics				
Severity of stenosis, %				
<50	3 (6)	0 (0)	0.06	
50–69	14 (29)	2 (9)		
≥70	31 (65)	20 (91)		
HIP	43 (90)	16 (73)	0.07	
CEUS grade				
Low				
0	0 (0)	5 (23)	<0.0001	
1	4 (8)	9 (41)		
High				
2	26 (54)	7 (32)		
3	18 (38)	1 (4)		
IPH score	0.43±0.19	0.37±0.22	0.38	
Neovessel density, /mm ²	56.3±43.2	17.7±15.0	<0.0001	
AHA classification				
V	3 (6)	3 (14)	0.31	
VI	44 (94)	19 (86)		
Treatments				
Statins				
Strong statin use	35 (73)	15 (68)	0.82	
Regular statin use	7 (15)	3 (14)		
No statin	6 (12)	4 (18)		
Antiplatelet therapy				
Aspirin monotherapy	25 (52)	13 (59)	0.40	
Clopidogrel monotherapy	8 (17)	1 (5)		
Cilostazol monotherapy	2 (4)	1 (5)		
Ticlopidine monotherapy	0 (0)	1 (5)		
Dual-antiplatelet therapy	13 (27)	6 (27)		

Data are given as mean±SD or number (percentage). P values are for comparison of symptomatic and asymptomatic groups. AHA indicates American Heart Association; CEUS, contrast-enhanced ultrasound; HIP, high-signal-intensity plaque; IPH, intraplaque hemorrhage.

OH) for 24 to 48 hours. They were then decalcified in EDTA for 1 week, after which 3-mm thick transverse tissue slices of the carotid artery were prepared. Paraffin-embedded sections of CEA specimens were stained with hematoxylin-eosin and Masson trichrome stains. Plaque morphological features were evaluated according to the American Heart Association classification of atherosclerotic plaques.⁷

Immunohistochemical evaluation with glycophorin A, which identifies red blood cell membranes, was performed to identify IPH.

Histological examinations were performed by an experienced pathologist (H.I.U.) who was blinded to the MR imaging and CEUS findings. As an index of the degree of IPH, the IPH score was calculated as the ratio of the glycophorin A-positive area/the whole plaque area.¹ Neovessel density

(per square millimeter) positive for von Willebrand factor in the plaque was counted in the shoulder of the most stenotic lesion. Discordance between CEUS assessment and IPN calculation may have occurred because we assessed the contrast effect of the whole plaque using CEUS and calculated the density of neovessels in the plaque shoulder of CEA specimens. However, our previous study² showed that the contrast occurred mainly in the plaque shoulders, with a significant correlation with the density of the neovessels in the pathophysiological study.

Statistical Analysis

JMP 9.0.2 software (SAS Institute, Cary, NC) was used for statistical analysis. Descriptive characteristics of all variables

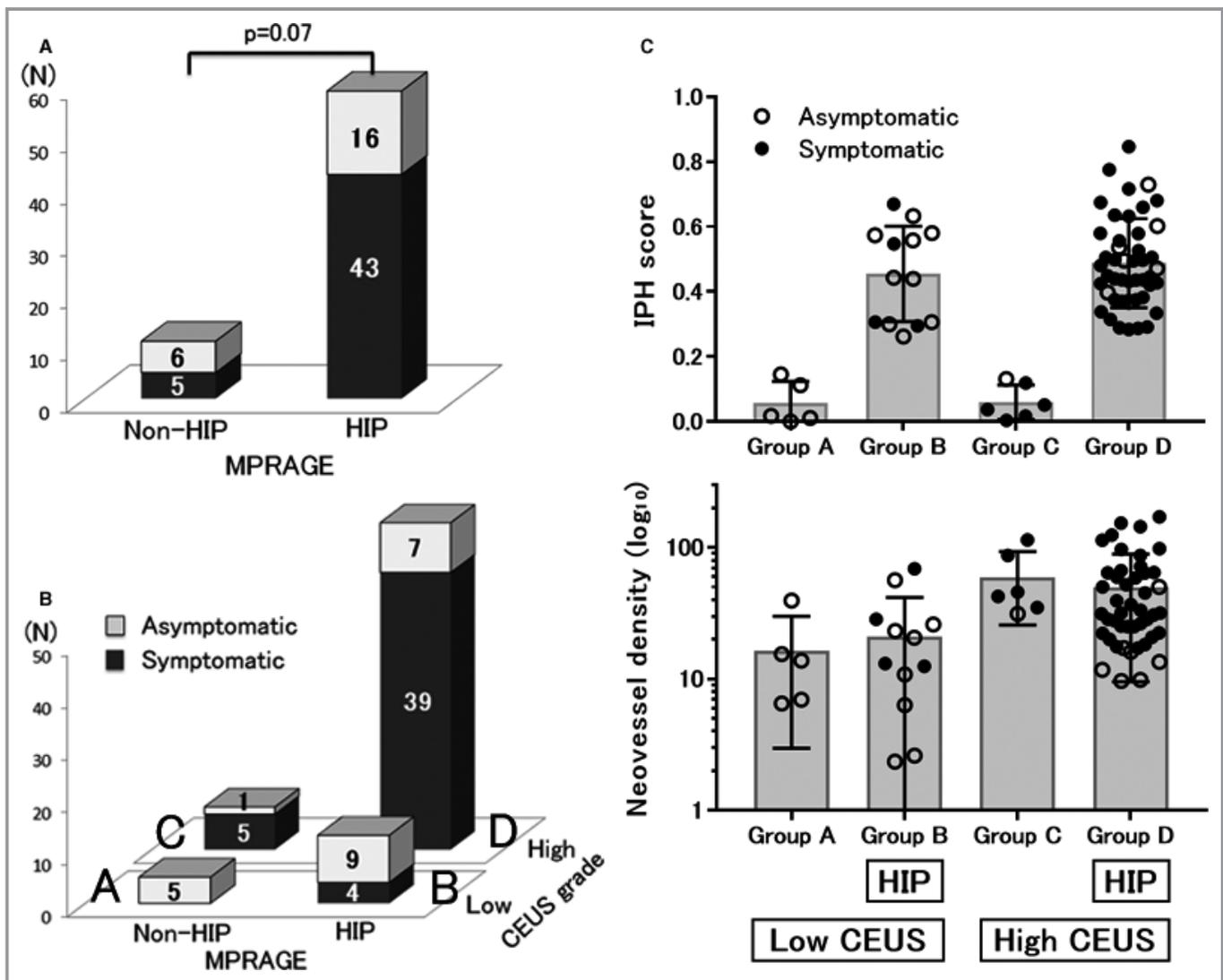


Figure 1. A and B, Magnetization-prepared rapid acquisition with gradient echo (MPRAGE) and contrast-enhanced ultrasound (CEUS) findings of plaques. C, Intraplaque hemorrhage (IPH) score and neovessel density for each group (groups A–D in B). HIP indicates high-signal-intensity plaque.

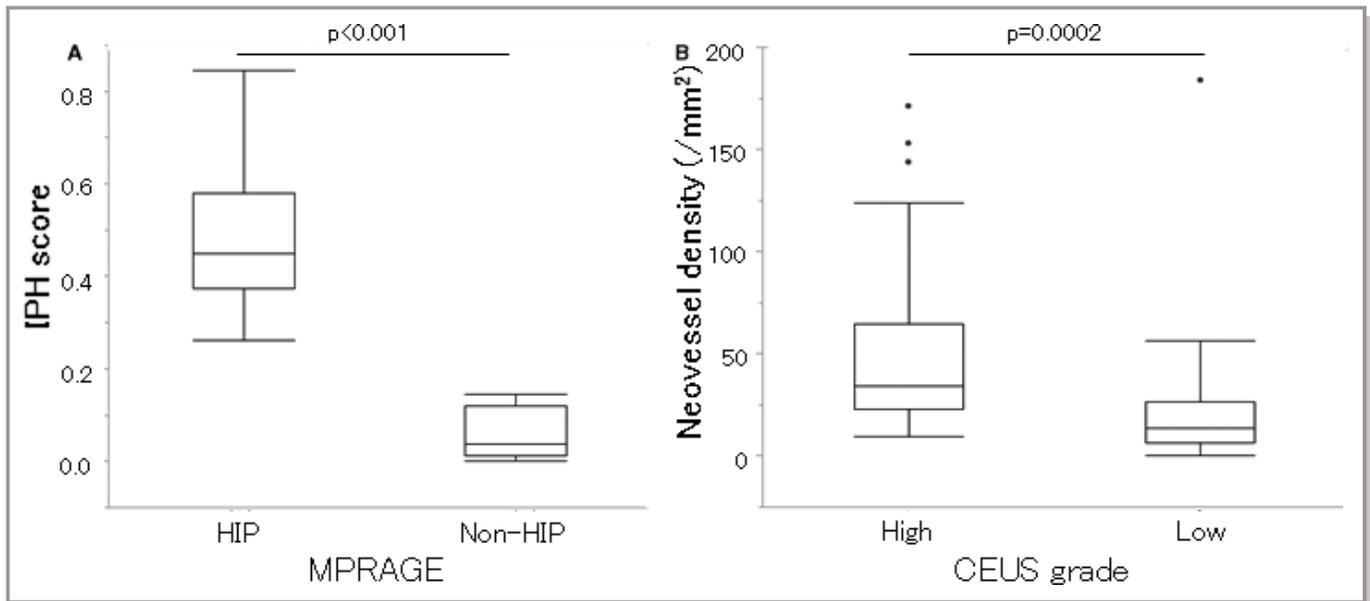


Figure 2. A, Box plot shows the intraplaque hemorrhage (IPH) score of plaques in high-signal-intensity plaque (HIP) and non-HIP groups. B, Box plots show the neovessel density of plaques in the high- and low-contrast effect groups of contrast-enhanced ultrasound (CEUS). The top border of the box shows the 75th percentile, the whisker shows the range of the data, and the horizontal line in the box shows the median. MPRAGE indicates magnetization-prepared rapid acquisition with gradient echo.

are expressed as means \pm SDs for continuous variables and as percentages for categorical variables. Statistical analysis was performed using the Wilcoxon rank sum test, the χ^2 test, or Fisher's exact test. $P < 0.05$ indicated statistical significance. The intrarater agreement of CEUS grading between the 2 observers was calculated using the κ statistic. Receiver operating characteristic curves were generated to compare the diagnostic value of detecting symptomatic plaques using MPRAGE and CEUS. The areas under the curve were calculated and compared with MPRAGE alone.

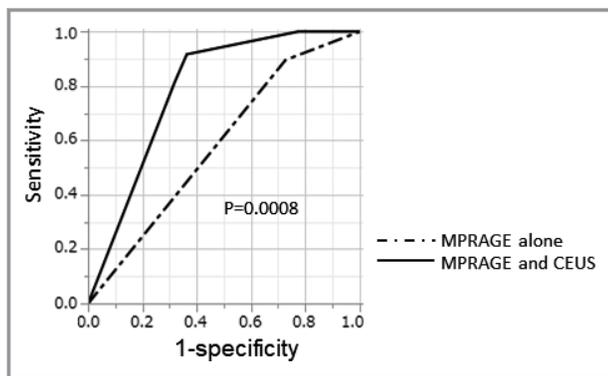


Figure 3. The receiver operating characteristic curve for detecting symptomatic plaques showed that the combined use of magnetization-prepared rapid acquisition with gradient echo (MPRAGE) and contrast-enhanced ultrasound (CEUS) was significantly more effective (area under the curve [AUC], 0.79; $P < 0.0001$) than MPRAGE alone (AUC, 0.58; $P = 0.07$) ($P = 0.0008$).

Results

Seventy-one consecutive patients were enrolled, although one patient was later excluded because of CEA tissue sample failure. The demographic data of the study group are presented in the Table. Among the 70 plaques, 59 were classified as HIPs (43 were symptomatic), and 11 were classified as non-HIPs (5 were symptomatic) (Figure 1A). Symptoms were classified as TIA in 8 patients (17%), amaurosis fugax in 9 patients (18%), and cerebral infarction in 31 patients (65%). In symptomatic patients, the median time intervals between stroke and MRI and CEUS performance were 29 and 37 days, respectively. (The median time interval between CEUS and MRI was 13 days.) Histopathological examination revealed that the IPH score was higher in the HIP than in the non-HIP group (0.48 ± 0.14 versus 0.06 ± 0.06 , respectively; $P < 0.001$) (Figure 2A). On the basis of CEUS images, 52 plaques were classified as being in the high-contrast effect group, and the other 18 were in the low-contrast effect group. A correlation was observed between the CEUS grade and symptoms ($P < 0.0001$). Histopathologically, neovessel density was greater in the high-contrast than in the low-contrast effect group ($50.5 \pm 39.0/\text{mm}^2$ versus $26.0 \pm 41.9/\text{mm}^2$, respectively; $P = 0.0002$) (Figure 2B).

When the groups were reclassified according to the grade of contrast effect in addition to the MRI findings (Figure 1B), all 5 symptomatic non-HIPs were classified into the high-contrast effect group (Figure 1B, black column in group C).

The receiver operating characteristic curve for detecting symptomatic plaques showed that the combined use of MPRAGE and CEUS was significantly more effective (area under the curve, 0.79; $P<0.0001$) than MPRAGE alone (area under the curve, 0.58; $P=0.07$) ($P=0.0008$) (Figure 3).

We observed compatibility between the histopathological findings and classification based on MRI and CEUS findings (groups A–D in Figure 1B and 1C). All 5 symptomatic non-HIPs (Figure 1C, group C) had extensive IPN (mean density, $64.9\pm 34.2/\text{mm}^2$) but only a small amount of IPH (mean score, 0.045 ± 0.04) in addition to a large necrotic core, active inflammation, and a thin/ruptured fibrous cap. These histological findings of most of all symptomatic cases (94%) were compatible with type VI of the conventional American Heart Association classification of atherosclerosis.⁷ Figure 4 shows a representative case.

Discussion

Using CEUS, we characterized the 5 symptomatic non-HIPs in this study as having extensive neovascularization. Histopathologically, these plaques showed extensive IPN but only a small amount of IPH.

We also showed an association between HIPs and pathological IPH, confirming that MPRAGE is useful for detecting IPH. MPRAGE, however, could not differentiate symptomatic plaques, as shown in Figure 1A. T1-weighted, magnetization-prepared, 3-dimensional gradient echo sequencing, including MPRAGE, is widely used and is superior for distinguishing plaques containing IPH.^{1,8} Saito et al,⁹ however, reported that lipid-rich plaques sometimes show isointensity or hypointensity on MPRAGE and cannot be discriminated from stable fibrous plaques, which may result in underestimating plaque vulnerability.

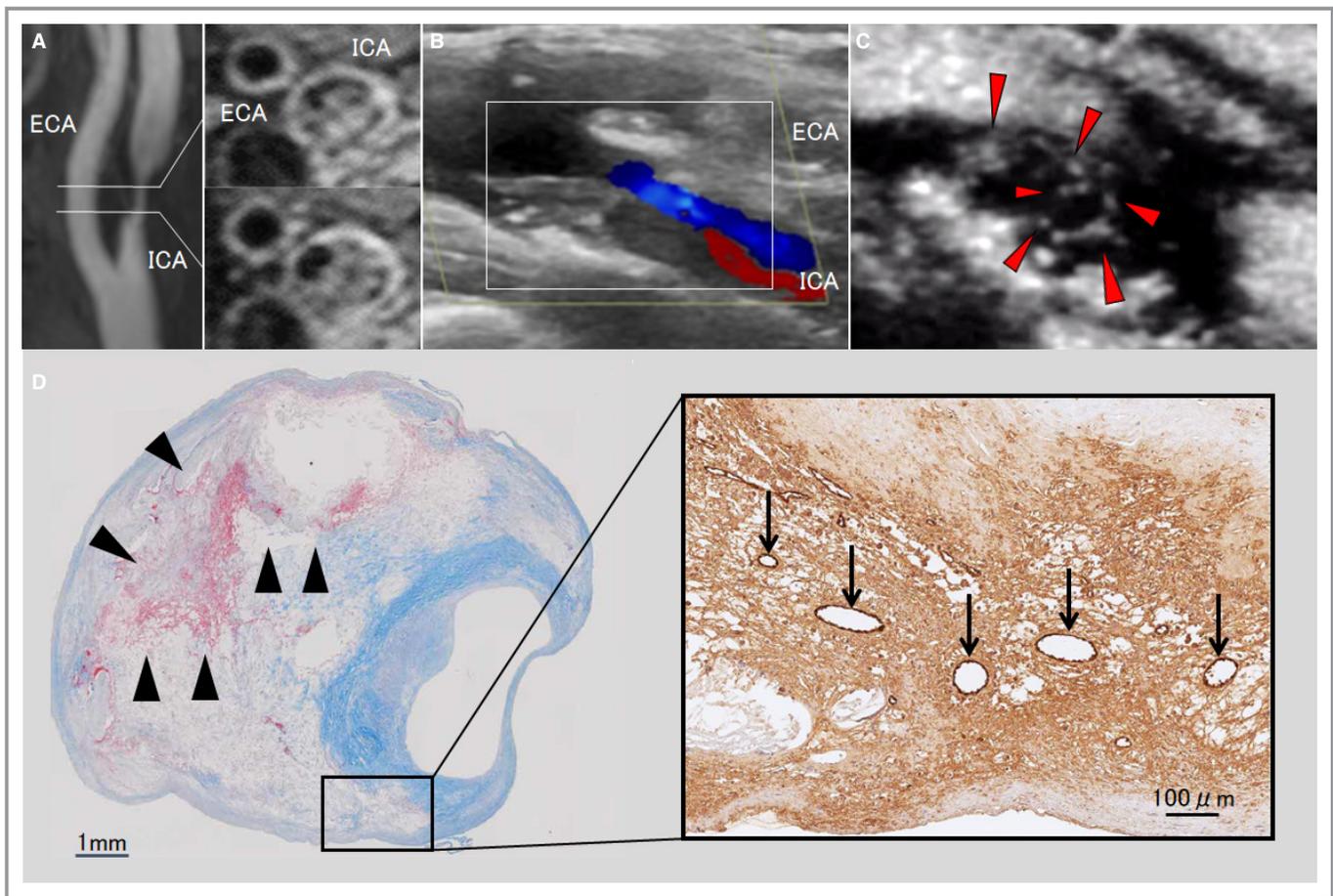


Figure 4. A case of amaurosis fugax refractory to medical treatment with symptomatic non-high-signal-intensity plaque (HIP) on magnetization-prepared rapid acquisition with gradient echo (MPRAGE) with a high-contrast effect on contrast-enhanced ultrasound (CEUS). **A** and **B**, Magnetic resonance angiography (**A**) and long-axis color Doppler ultrasound (**B**) images show a severely stenotic plaque in the internal carotid artery (ICA). **A**, MPRAGE images (axial) show non-HIP. **C**, With CEUS (shown as the square in **B**), many microbubbles are recognized in the plaque (red arrowheads), which is classified as grade 3. **D**, Photomicrograph of a carotid endarterectomy specimen with a large necrotic core (high magnification of the square) shows extensive neovascularization (black arrows) and a small amount of intraplaque hemorrhage (black arrowheads). ECA indicates external carotid artery.

Neovascularization is a prominent feature of the early stages of atherosclerotic plaque development, as shown in previous histopathological studies.¹⁰ Neovascularization also indicates plaque vulnerability because neovessels are fragile and tend to collapse, resulting in IPH¹⁰ and, with the release of chemical mediators into the plaques, the appearance of inflammatory cells.¹¹ During this early stage of destabilization, before the collapse of neovessels results in IPH, we may fail to detect vulnerability using only MPRAGE. In contrast, CEUS can detect neovessels in real time with less invasiveness.² CEUS also allows easy follow-up evaluation before progression to IPH. Analysis of large atherosclerotic carotid plaque biobanks has also shown that the predicted stroke risk is related to high neovessel density, but not IPH,¹² which suggests the superiority of CEUS for early detection of vulnerable plaques.

Although CEUS and MRI have advantages and specific limitations for clinical application, these 2 methods are likely to provide different, but complementary, information on plaque character. Shimada et al¹³ showed that CEUS findings in plaque were related to those detected by 3-dimensional fast spin echo T1-weighted MRI. We showed the superiority of the combined use of MPRAGE and CEUS, which was confirmed histopathologically and is reasonable for following the destabilization process of atherosclerosis. In clinical practice, CEUS may be more available to evaluate plaque vulnerability for screening, considering cost and facilities, than MRI as a reasonable workflow that takes into consideration the atherosclerotic process, especially in outpatient clinics. To our knowledge, the combined use of the 2 modalities for evaluating plaque vulnerability has not been previously applied or confirmed histopathologically, although these modalities are readily available in clinical practice.

The limitations of this study include the small sample size and the small number of patients with symptomatic non-HIPs. During the study period, between July 2010 and June 2014, a total of 240 patients with internal carotid stenosis underwent CEA. However, we did not include the following patients: (1) those undergoing emergency CEA, (2) those without acquisition of informed consent, (3) those without evaluation of MPRAGE, (4) those without an ultrasound examination, and (5) those with severely calcified plaques who did not undergo CEUS. All these factors explain the seemingly small patient population in our study. These are preliminary data to be confirmed in a larger study with a prospective design. It may also include selection bias of plaques evaluable by CEUS and inclusion of many high-risk patients who underwent CEA to confirm histopathological findings. There were also time intervals between the stroke/TIA onset in symptomatic patients and the CEUS and MRI performance because of referral delay and patient and/or device availability. To our

knowledge, although plaque vulnerability may be subject to rapid change, longitudinal MRI data of carotid plaque suggest that high-risk elements (eg, plaque hemorrhage) do not change significantly over a 1-year period.¹⁴ It is unclear how this interval might have affected our results.

In conclusion, we detected vulnerable plaques with more accuracy using CEUS to find IPN and MPRAGE imaging to find IPH. Complementary use of these 2 modalities could be useful for evaluating plaque vulnerability, consistent with the growth and destabilization process of atherosclerotic plaques. These are preliminary data to be confirmed in a larger study with a prospective design.

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Disclosures

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