Background: Contrast-enhanced ultrasound (CEUS) in the carotid artery has potential as a technique for imaging plaque neovascularization. This study examined whether CEUS could provide information on the severity and instability of coronary artery disease (CAD).

Methods and Results: A total of 304 patients with CAD and carotid plaque underwent CEUS examination of the carotid artery. Intraplaque neovascularization was identified on the basis of microbubbles within the plaque and graded as: G0, not visible; G1, moderate; or G2, extensive microbubbles. The complexity and extent of the coronary lesions were assessed angiographically. A higher grade of CEUS-assessed plaque neovascularization of the carotid artery was associated significantly with greater complexity ($p=0.48$ by Spearman’s rank correlation test) and extent ($p=0.51$) of coronary lesions. G2 plaque neovascularization was a risk for acute coronary syndrome, independent of traditional risk factors (odds ratio 1.91, 95% confidence interval 1.04–3.53, $P<0.01$). Subgroup analysis showed that carotid CEUS-assessed neovascularization regressed in 12 (46%) of 26 plaques in patients during 6 months of statin treatment, whereas regression occurred in 2 (14%) of 14 plaques in patients not taking a statin ($P=0.04$, Chi-square test).

Conclusions: CEUS examination of the carotid artery may provide valuable information on the severity and instability of CAD and also the efficacy of antiatherosclerotic treatment. (Circ J 2013; 77: 1499–1507)

Key Words: Acute coronary syndrome; Contrast-enhanced ultrasound; Coronary artery disease; Plaque neovascularization

Increased neovascularization in atherosclerotic plaque has been shown to be associated with plaque progression and instability, leading to atherosclerotic occlusive cardiovascular events. Contrast-enhanced ultrasound (CEUS) is an emerging valuable tool for visualizing plaque neovascularization in the carotid artery, with the properties of contrast agent microbubbles, making them act as pure intravascular tracers. CEUS has the advantages of being a simple and minimally invasive in vivo technique. The usefulness and reliability of CEUS have been validated by previous studies in animals and humans that showed the degree of plaque neovascularization assessed by CEUS correlated strongly with histological density of neovessels. CEUS also showed a greater extent of plaque neovascularization of the carotid artery in symptomatic patients with previous cerebrovascular diseases.

A previous study indicated that plaque instability is not merely a local vascular occurrence, but rather exists simultaneously at multiple sites in the systemic vascular bed. It is therefore possible that instability of coronary plaque could be assessed by evaluating plaque neovascularization in the carotid arteries. This study therefore examined whether CEUS of the carotid artery provided information on the severity and instability of coronary artery disease (CAD) and also assessed the efficacy of statin treatment in patients with CAD.
Table 1. Baseline Clinical Characteristics of the Study Patients With Coronary Artery Disease

| Overall (n=304) | Grade of plaque neovascularization assessed by carotid contrast-enhanced ultrasound |
|----------------|----------------------------------------------------------------------------------|
|                | 0 (n=141) | 1 (n=93) | 2 (n=70) | P value |
| Age (years)    | 68.7±10   | 68.2±11  | 68.7±11  | 69.5±9  | 0.70   |
| Sex, male n (%)| 245 (81)  | 109 (77) | 78 (84)  | 58 (82) | 0.39   |
| Current smoking, n (%) | 115 (38) | 45 (32) | 38 (41) | 32 (46) | 0.12   |
| Hypertension, n (%) | 153 (50) | 65 (46) | 45 (48) | 43 (61) | 0.10   |
| Diabetes mellitus, n (%) | 138 (45) | 52 (37) | 48 (52)* | 38 (54)* | 0.02   |
| Body mass index (kg/m²) | 23.7±3.0 | 24±3.0 | 23.6±3.1 | 23.4±3.0 | 0.44   |
| Systolic BP (mmHg) | 138±13    | 138±14   | 138±14   | 141±12   | 0.36   |
| LDL-C (mg/dl)   | 109±33    | 104±32   | 109±32   | 118±34*  | 0.02   |
| HDL-C (mg/dl)   | 45±13     | 47±13    | 43±11*   | 44±13*   | 0.03   |
| Triglycerides (mg/dl) | 126 (95,162) | 128 (96,161) | 122 (88,160) | 127 (98,169) | 0.17   |
| Hemoglobin A1c (%) | 5.8 (5.4, 6.4) | 5.9 (5.5, 6.6) | 5.8 (5.5, 6.3) | 5.7 (5.4, 6.5) | 0.69   |
| CRP (mg/L)      | 0.9 (0.3, 3.3) | 0.6 (0.2, 1.4) | 0.8 (0.3, 1.8)* | 3.8 (1.0, 9.0)* | 0.004  |
| Troponin T (ng/ml) | 0.01 (0.006, 0.06) | 0.01 (0.003, 0.008) | 0.02 (0.014, 0.028)* | 0.29 (0.122, 0.783)* | <0.0001 |
| ACS, n (%)      | 84 (28)   | 24 (17)  | 28 (30)  | 32 (34)* | <0.001 |
| Previous MI, n (%) | 59 (19)  | 33 (23)  | 14 (15)  | 13 (19)  | 0.20   |
| CTO lesion, n (%) | 49 (16)  | 15 (12)  | 15 (10)  | 19 (27)* | 0.009  |
| PAD, n (%)      | 37 (12)   | 11 (8)   | 9 (10)   | 17 (24)* | 0.002  |
| History of CABG, n (%) | 17 (6)   | 6 (4)    | 6 (6)    | 5 (7)    | 0.63   |
| History of stroke | 25 (8)   | 11 (8)   | 9 (10)   | 6 (9)    | 0.97   |
| Maximum IMT (mm) | 2.5±1.0   | 2.2±0.7  | 2.7±1.0* | 3.1±1.2* | <0.001 |
| Ankle-brachial index | 1.10±0.13 | 1.12±0.11 | 1.11±0.12 | 1.07±0.16* | 0.02   |
| PWV (cm/s)      | 1,731±372 | 1,710±398 | 1,746±318 | 1,753±386 | 0.64   |

Medication use, n (%)

| Statin               | 161 (53) | 84 (71) | 48 (52) | 29 (40)* | 0.02   |
| ACEI/ARB             | 120 (39) | 60 (43) | 33 (35) | 27 (39)  | 0.55   |
| β-blocker            | 42 (14)  | 24 (17) | 10 (11) | 8 (11)   | 0.32   |
| Ca-blocker           | 100 (33) | 45 (32) | 26 (28) | 29 (41)  | 0.18   |
| Aspirin              | 304 (100)| 141 (100)| 93 (100)| 79 (100) | –      |
| Thienopyridine       | 244 (80) | 105 (74) | 77 (83) | 62 (89)  | 0.06   |
| Sulfonylurea         | 39 (13)  | 15 (11) | 12 (13) | 12 (13)  | 0.41   |
| Biguanide            | 21 (7)   | 11 (8)  | 3 (3)   | 7 (10)   | 0.20   |
| Insulin              | 11 (4)   | 5 (4)   | 4 (4)   | 2 (3)    | 0.89   |
| Diuretics            | 56 (18)  | 22 (16) | 19 (20) | 15 (21)  | 0.49   |

*P<0.05 vs. patients in grade 0. Data are expressed as the mean value ± SD, median and range (25th and 75th percentiles) or the number (%) of patients.

ACEI, angiotensin converting enzyme inhibitor; ACS, acute coronary syndrome; ARB, angiotensin II receptor blocker; BP, blood pressure; CABG, coronary artery bypass graft surgery; CRP, C-reactive protein; CTO, chronic total occlusion of coronary artery; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; IMT, intima media thickness; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PAD, peripheral artery disease; PWV, pulse wave velocity.

Methods

Study Patients

We initially screened 1,369 consecutively enrolled patients with CAD who underwent coronary angiography and carotid ultrasound in the cardiology section of Yamanashi University Hospital from February 2010 to June 2012. We included patients who had both carotid intima-media thickness (IMT) ≥1.1 mm and organic stenosis >70% of the diameter of at least 1 major coronary artery or ≤70% after spontaneous re-ocanization of the culprit lesion when patients had acute coronary syndrome (ACS). The exclusion criteria were: (1) allergies to the ultrasound contrast agent; (2) previous carotid surgery or angioplasty; (3) stroke, cardiogenic shock, pulmonary edema, major surgery, trauma, or serious infectious disease within 4 weeks prior to enrollment; (4) neoplasm, significant hepatic or intestinal inflammatory disease; (5) chronic renal failure or serum creatinine level >2.5 mg/dl or congestive heart failure (NYHA classification ≥III) at admission; (6) other serious diseases; and (7) age >80 years. ACS was diagnosed by the presence of acute ischemic symptoms lasting ≥20 min within the 48 h prior to admission to hospital, and ECG changes consistent with ACS. The presence of ACS was also confirmed by coronary angiography in all patients. Acute myocardial infarction was diagnosed when creatine kinase-MB levels increased to at least twice the upper limit of normal (3.5 ng/ml) or when troponin T levels were >0.1 ng/ml. The study finally included 304 patients who fulfilled the inclusion and exclusion criteria. The baseline clinical characteristics and medication use are shown in Table 1. Written informed consent was given by all patients before the study, which was approved by the institution’s ethics committee. The investigation conformed to the principles
Carotid CEUS and CAD

with a maximum of 100, which represented the percentage of the coronary luminal surface area containing atheroma. The inter- and intraobserver variability for repeated measurements of this score was 5.1% and 3.4%, respectively.

A diseased coronary artery was defined as a major coronary artery with an organic stenosis >70%.

Standard US Study in the Carotid Artery
A standard carotid US examination was performed for all study patients at the screening visit using a 11.0-MHz, linear-array transducer (Prosound α10, HITACHI-ALOKA Medical, Tokyo, Japan), as described in our previous reports. Briefly, longitudinal images of each carotid artery were used to measure IMT, defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface. Atherosclerotic plaques were defined as lesions with a focal IMT ≥1.1 mm that protruded from the vessel wall into the lumen. In each patient, we selected plaque with the maximum IMT (plaque-maxIMT), defined as the greatest axial thickness of all plaques at baseline.

CEUS in the Carotid Artery
Carotid CEUS studies were performed using same machine as for the standard studies and a UST-5412 probe with a transmission frequency of 4.5–7.5 MHz. These scans were carried out and analyzed by 2 researchers (T.N., I.T.) who were blinded to the history and characteristics of the patients. Fol-
proximately 2 min per plaque, depending on the number of plaques in each patient. Intraplaque neovascularization was ≥ with a focal IMT ≥1.1 mm of the left and right side for approximately 2 min per plaque, depending on the number of plaques in each patient. Intraplaque neovascularization was identified by rapid movement of the echogenic reflectors of microbubbles within the plaque and graded as: G0, no visible microbubbles within the plaque; G1, moderate microbubbles confined to the shoulder and/or adventitial side of the plaque; or G2, extensive microbubbles throughout the plaque. In each patient, plaque with the highest grade of intraplaque neovascularization was selected from all the carotid plaques with IMT ≥1.1 mm and used for the majority of the analyses.

Follow-up Study
The standard US and CEUS studies of the carotid artery were repeated 6 months (2nd study) after enrollment (1st study) in a subgroup of 40 patients with stable CAD who had not received statin treatment for more than 6 months prior to enrollment. In accordance with the guideline of the Japanese Atherosclerotic Society, statin treatment was started in 26 of the 40 patients after the 1st study because they had low-density lipoprotein cholesterol (LDL-C) levels ≥100 mg/dl. Statins were not prescribed during the 6-month follow-up period in the remaining 14 patients, who had LDL-C levels <100 mg/dl at enrollment. Also, a repeat evaluation of each patient’s atherosclerotic risk profile was carried out 6 months after the 1st study. Plaque with the highest grade of intraplaque neovascularization at the 1st CEUS study was selected to monitor the changes in the grade of plaque neovascularization between the 2 studies. To ensure that the same targeted plaques were measured at the 2nd CEUS study, the exact distance of the targeted plaque from the bifurcation of the common carotid artery or a site of arterial calcification was recorded as a landmark in each patient at the 1st CEUS study. The image settings were the same for both studies.

Magnetic Resonance Imaging (MRI) of Carotid Plaques
MRI can assess plaque characteristics, including vulnerability. To validate whether a higher grade of CEUS-assessed neovascularization reflects a lipid-rich, vulnerable condition, we evaluated the same carotid plaques in some patients with both MRI and CEUS. The carotid artery was imaged with a 1.5-T MR scanner (Intera 1.5T; Philips, Eindhoven, the Netherlands) and phase-array surface coils in 7 patients with both MRI and CEUS. The carotid artery was imaged with a 1.5-T MR scanner (Intera 1.5T; Philips, Eindhoven, the Netherlands) and phase-array surface coils in 7 patients with both MRI and CEUS. The carotid artery was imaged with a 1.5-T MR scanner (Intera 1.5T; Philips, Eindhoven, the Netherlands) and phase-array surface coils in 7 patients with both MRI and CEUS. The carotid artery was imaged with a 1.5-T MR scanner (Intera 1.5T; Philips, Eindhoven, the Netherlands) and phase-array surface coils in 7 patients with both MRI and CEUS. The carotid artery was imaged with a 1.5-T MR scanner (Intera 1.5T; Philips, Eindhoven, the Netherlands) and phase-array surface coils in 7 patients with both MRI and CEUS.

Laboratory Examination and Other Measurements
Venous blood samples were obtained after a 12-h overnight fast at the time of the carotid US examination. High-sensitivity C-reactive protein (CRP) levels were measured by rate nephelometry (Dade Behring, Marburg, Germany), while serum total cholesterol and triglyceride concentrations were measured enzymatically. Cardiac troponin T levels were measured on an ECLIusis instrument (Roche Diagnostics, Tokyo) using a commercially available immunoassay. The ankle-brachial index and carotid-femoral pulse wave velocity were measured using a well-validated noninvasive device (OMRON COLIN BP-RPE III form III, Tokyo, Japan).

Statistical Analysis
Data are expressed as either the mean value±SD, median and range (25th and 75th percentiles), or frequencies (%). The Shapiro-Wilk test showed that the levels of triglyceride, he-
Grade of CEUS-assessed plaque neovascularization was assessed from the plaques examined for further analysis. A higher grade of CEUS-assessed plaque neovascularization was noted in 239 plaques (49%), G1 in 156 plaques (32%), and G2 in 94 plaques (19%). The average grade of plaque neovascularization per patient was 1.13. Representative intraplaque neovascularization assessed by CEUS is shown in Figure 1 and Movies S1–S3 (Supplementary Files 1–3). In each patient, the plaque with the highest grade of CEUS-assessed intraplaque neovascularization was selected from the plaques examined for further analysis. A higher grade of CEUS-assessed plaque neovascularization was associated with higher levels of low-density lipoprotein cholesterol (LDL-C), CRP, troponin T, and carotid maxIMT, and lower high-density lipoprotein cholesterol (HDL-C) levels and ankle-brachial index, and higher prevalence of diabetes, ACS on hospital admission and peripheral artery disease (Table 1, P<0.05 for all). The prevalence of patients who had been taking a statin at enrollment was higher in the lower grades of plaque neovascularization (G0, 1, and 2=71%, 52%, and 40%, respectively). [G0 vs. G2, P=0.02 by Chi-square test] (Table 1).

Relationship Between the Grade of CEUS-Assessed Plaque Neovascularization and Complexity and Extent of Coronary Lesions

The 34 patients with a previous history of coronary intervention or coronary bypass surgery were excluded from the angiographic analysis to avoid artificial bias. In the remaining 270 patients, 118 patients had 1 complex lesion, and 57 patients had multiple complex lesions. Single-vessel disease (1 major coronary artery diseased with organic stenosis >70%) was identified in 92 patients, 2-vessel disease in 84 patients and 3-vessel disease in 62 patients. As shown in Figure 2, Spearman’s rank correlation test showed that the grade of CEUS-assessed plaque neovascularization had a significant correlation with coronary extent score (ρ=0.48, P<0.001), number of complex coronary lesions (ρ=0.51, P<0.001), and number of diseased coronary arteries (ρ=0.48, P<0.001). Also, the carotid maxIMT had a significant correlation with these coronary indices using linear regression analysis (r=0.20, 0.14, and 0.18, respectively, P<0.03 in all). Chronic total occlusion of coronary lesions was more prevalent in patients with G2 plaque neovascularization than in those with G0 plaque neovascularization (27% vs. 12%, respectively, P<0.01 by Chi-square test) (Table 1).

Association Between ACS and Grade of CEUS-Assessed Plaque Neovascularization

In total, 84 patients had ACS at the enrollment. In the univariate logistic regression analysis, patients with ACS at hospital admission showed a significant association with age (odds ratio (OR) 1.03), diabetes (OR 1.68), LDL-C (OR 1.02), CRP
peared hyperintense to isointense on T1 W images, whereas G0 plaque neovascularization appeared low intense on T1 W and T2 W images (Figure 4).

Reproducibility of CEUS-Assessed Plaque Neovascularization

Intra- and interobserver agreement assessed by Cohen $\kappa$ was determined in 100 carotid arteries graded for plaque neovascularization. The respective agreement was 0.82 and 0.84 (κ coefficient) for the presence of G2 grade neovascularization, 0.80 and 0.81 for the presence of G1 grade neovascularization, and 0.84 and 0.91 for G0 grade neovascularization.

Discussion

This study demonstrated that a higher grade of carotid plaque neovascularization assessed using CEUS was associated with more complex and extensive coronary lesions and a higher prevalence of multivessel CAD and chronic total occlusion of coronary lesions. It is known that angiographically complex coronary lesions represent either vulnerable plaques prone to disruption or truly disrupted plaques. In fact, the present study demonstrated that G2 plaque neovascularization assessed by CEUS was associated significantly with ACS at hospital admission. Moreover, CEUS-assessed plaque neovascularization significantly correlated with troponin T levels at admission in patients with ACS. Therefore, this study is the first to reveal a significant association of carotid CEUS-assessed plaque neovascularization with the severity and instability of CAD in patients with CAD. In addition, the study showed that patients taking a statin at enrollment had a lower grade of the carotid CEUS-assessed plaque neovascularization. Furthermore, statin

| Table 3. Changes in Lipids and Other Parameters After 6 Months With or Without Statin Treatment |
|-----------------------------------------------|
| Statin (+) (n=26) | Statin (–) (n=14) | P value |
|------------------|------------------|---------|
| **LDL-C (mg/dl)** |                   |         |
| Baseline         | 139±29           | 92±28   | <0.01 |
| 6 months         | 91±23*           | 108±17  | 0.01  |
| % change from baseline | −30.5±18       | 15.1±26 | <0.0001 |
| **Triglycerides (mg/dl)** |               |         |
| Baseline         | 129 (94, 203)    | 99 (88, 117) | 0.04 |
| 6 months         | 119 (105, 179)   | 116 (88, 155) | 0.74 |
| % change from baseline | −7.7±53.5       | 16.9±93.6 | 0.29 |
| **HDL-C (mg/dl)** |                   |         |
| Baseline         | 42.1±11.4        | 48.3±13.2 | 0.13 |
| 6 months         | 44.3±15.5        | 43.1±9.3  | 0.78 |
| % change from baseline | 8.9±40.7        | −7.8±21.7 | 0.16 |
| **CRP (mg/L)**   |                   |         |
| Baseline         | 0.47 (0.08, 0.87) | 0.18 (0.09, 0.37) | 0.39 |
| 6 months         | 0.06 (0.02, 0.10)* | 0.09 (0.04, 0.26) | 0.10 |
| % change from baseline | −34.4±61        | −8±57    | 0.03 |
| **Maximum IMT (mm)** |             |         |
| Baseline         | 2.1±0.81         | 2.5±0.57 | 0.10 |
| 6 months         | 2.2±0.86         | 2.6±0.64 | 0.13 |
| % change from baseline | 5.7±14.8       | 4.8±18.7 | 0.87 |

*P<0.05 vs. baseline values. Abbreviations as in Table 1.
Carotid CEUS and CAD

CEUS imaging might reflect the presence and degree of neovascularization of carotid plaque. Other noninvasive or invasive imaging modalities might be able to assess neovascularization in the carotid and coronary arteries in the clinical setting. Dynamic contrast-enhanced MRI can quantitatively measure neovascularity in carotid plaques and optical coherence tomography can identify plaque neovascularization in the coronary artery. Further studies are warranted to determine the predictive values of these imaging modalities for patients with CAD at high risk.

Study Limitations
First, the study had the disadvantage of relatively high subjectivity because of the use of semiquantitative image analysis. Some reports have used computer-assisted quantitative image analyses of carotid plaque neovascularization, but it is not available for general clinical practice. Second, the results of the effects of statin are preliminary because they were not obtained from a comparative study with randomized allocation to statin treatment. Third, it is possible that significant neovascularization in some carotid plaques may have been overlooked because of technical reasons or the presence of acoustic shadows in echogenic or calcified plaques. Fourth, plaque location treatment after enrollment was associated with an improvement in carotid CEUS-assessed plaque neovascularization. This finding is consistent with previous experimental studies that showed neovascularization in atherosclerotic plaque was reduced by statin treatment. On the basis of previous reports, statin treatment may reduce neovascularization via multiple mechanisms (e.g., antiinflammatory and antioxidant actions and reduction in the production of angiogenic factors including vascular endothelial growth factor as well as reduction in LDL-C levels). Thus, carotid CEUS imaging might identify changes in plaque neovascularization and thus monitor the therapeutic effects on plaque neovascularization of the carotid artery. However, a prospective study of antiatherosclerotic interventions using carotid CEUS in a larger number of patients is needed to confirm whether the technique is useful for assessing risk stratification of future coronary events and therapeutic efficacy.

The present MRI study showed that a high grade of the CEUS-assessed neovascularization was associated with hyperintense or isointense signals on T1 W images, reflecting a lipid-rich necrotic core classified according to MRI criteria, although only a limited number of patients were examined. In agreement with a previous study, this finding supports that CEUS imaging might reflect the presence and degree of neovascularization of carotid plaque.
Conclusions

CEUS of the carotid artery may provide valuable information on coronary risk status and the efficacy of anti-atherosclerotic treatment in patients with CAD.

Figure 4. Magnetic resonance (MR) imaging and the corresponding contrast-enhanced ultrasound (CEUS) images of carotid plaques. All the images show a region of the carotid artery near the bifurcation. (A) Representative case of grade 0 CEUS-assessed plaque neovascularization in the internal carotid artery. The red arrow indicates plaque in the internal carotid artery and the white arrow, the external carotid artery. The corresponding MR images of the plaque show hypointense signals on T1 W and TOF and isointense signals on T2 W, compared with the adjacent sternocleidomastoid muscle. (B) Representative case with grade 2 plaque neovascularization in the common carotid artery. The yellow arrows indicate microbubbles in the plaque and the red arrow indicates plaque. The corresponding MR images show isointense signals on T1 W and hyperintense signals on T2 W and TOF. (C) Plot of each plaque with CEUS-assessed grade of plaque neovascularization according to signal intensity of T1 W and T2 W. TOF, time-of-flight; T1 W, T2 W: T1- or T2-weighted.
Acknowledgments
This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology, Health, Tokyo, Japan (grants-in-aid for B2-19390209 and B-22390158).

References
1. Moreno PR, Purushothaman KR, Sirol M, Levy AP, Fuster V. Neovascularization in human atherosclerosis. Circulation 2006; 113: 2245 – 2252.
2. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, et al. Atherosclerotic plaque progression and vulnerability to rupture: Angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 2005; 25: 2054 – 2061.
3. Staub D, Schinkel AF, Coli S, Coli S, van der Steen AF, Reed JD, et al. Contrast-enhanced ultrasound imaging of the vasa vasorum: From early atherosclerosis to the identification of unstable plaques. JACC Cardiovasc Imaging 2010; 3: 761 – 771.
4. Feinstein SB. Contrast ultrasound imaging of the carotid artery vasa vasorum and atherosclerotic plaque neovascularization. J Am Coll Cardiol 2006; 48: 236 – 243.
5. Shallhoub J, Owen DR, Gauthier T, Monaco C, Leen EL, Davies AH. The use of contrast enhanced ultrasound in carotid arterial disease. Eur J Vasc Endovasc Surg 2010; 39: 381 – 387.
6. Coli S, Magnoni M, Sangiorgi G, Marrocco-Trischitta MM, Melisurgo G, Maurilio A, et al. Contrast-enhanced ultrasound imaging of intraplaque neovascularization in carotid arteries: Correlation with histology and plaque echogenicity. J Am Coll Cardiol 2008; 52: 223 – 230.
7. Hoogi A, Adam D, Hoffman A, Kern K, Zames S, Taitini D. Carotid plaque vulnerability: Quantification of neovascularization on contrast-enhanced ultrasound imaging with histopathologic correlation. Am J Roentgenol 2011; 196: 431 – 436.
8. Giannarelli C, Ibanez B, Cimmino G, Garcia Ruiz JM, Faita F, Bianchini E, et al. Contrast-enhanced ultrasound imaging detects intraplaque neovascularization in an experimental model of atherosclerosis. JACC Cardiovasc Imaging 2010; 3: 1256 – 1264.
9. Staub D, Patel MB, Tibrewala A, Ludden D, Johnson M, Espinosa P, et al. Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke 2010; 41: 41 – 47.
10. Huang PT, Chen CC, Aronow WS, Wang XT, Nair CR, Xue NY, et al. Assessment of neovascularization within carotid plaques in patients with ischemic stroke. World J Cardiol 2010; 2: 89 – 97.
11. Rothwell PM, Villagra R, Gibson R, Donders RC, Warlow CP. Evidence of a chronic systemic cause of instability of atherosclerotic plaques. Lancet 2000; 355: 19 – 24.
12. Hirano M, Nakamura T, Obata JE, Fujioka D, Saito Y, Kawabata K, et al. Early improvement in carotid plaque echogenicity by acarbose in patients with acute coronary syndromes. Circ J 2012; 76: 1452 – 1460.
13. Watanabe K, Sugiyaama S, Kugiyama K, Honda O, Fukushima H, Koga H, et al. Stabilization of carotid atheroma assessed by quantitative ultrasound analysis in non hypercholesterolemic patients with coronary artery disease. J Am Coll Cardiol 2005; 46: 2022 – 2030.
14. Ambrose J, Winters SL, Stein A, Eng A, Teichholz LE, Gorlin R, et al. Angiographic morphology and the pathogenesis of unstable angina pectoris. J Am Coll Cardiol 1985; 5: 699 – 716.
15. Sullivan DR, Marwick TH, Freedman SB. A new method of scoring coronary angiograms to reflect extent of coronary atherosclerosis and improve correlation with major risk factors. Am Heart J 1990; 119: 1262 – 1267.
16. Teramoto T, Sasaki J, Ueshima H, Egusa G, Kinoshita M, Shimamoto K, et al. 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.
17. Yuan C, Matusmori LM, Ferguson MS, Polissar NL, Echelard D, Ortiz G, et al. In vivo accuracy of multisperctral magnetic resonance imaging for identifying lipid-rich necrotic cores and intraplaque hemorrhage in advanced human carotid plaques. Circulation 2001; 104: 2051 – 2056.
18. Chen L, Chester MR, Redwood S, Huang J, Leatham E, Kaski JC. Angiographic stenosis progression and coronary events in patients with “stabilized” unstable angina. Circulation 1995; 91: 2319 – 2324.
19. Koutouzis M, Namkias A, Nikolaidis S, Tzavara V, Andrikopoulos V, Nikolau N, et al. Statin treated patients have reduced intraplaque angiogenesis in carotid endarterectomy specimens. Atherosclerosis 2007; 192: 457 – 463.
20. Kerwin W, Hooper A, Spilker M, Vicini P, Ferguson M, Hatsukami T, et al. Quantitative magnetic resonance imaging analysis of neovascular volume in carotid atherosclerotic plaque. Circulation 2003; 107: 851 – 856.
21. Kitabata H, Tanaka A, Kudo T, Takarada S, Kashiwagi M, Tsujioka H, et al. Relation of microchannel structure identified by optical coherence tomography to plaque vulnerability in patients with coronary artery disease. Am J Cardiol 2010; 105: 1673 – 1678.
22. Ohota M, Kawasaki M, Ismail TF, Hattori K, Serruys PW, Ozaki Y. A histological and clinical comparison of new and conventional integrated backscatter intravascular ultrasound (IB-VUS). Circ J 2012; 76: 1678 – 1686.
23. Solberg LA, Egggen DA. Localization and sequence of development of atherosclerotic lesions in the carotid and vertebral arteries. Circulation 1971; 43: 711 – 724.

Supplementary Files
Supplementary File 1
Movie S1. Representative case of Grade 0 intraplaque neovascularization assessed by contrast-enhanced ultrasound (CEUS). There are no visible microbubbles within the plaque.

Supplementary File 2
Movie S2. Representative case of Grade 1 intraplaque neovascularization assessed by contrast-enhanced ultrasound (CEUS). There are moderate microbubbles confined to the shoulder of the plaque.

Supplementary File 3
Movie S3. Representative case of Grade 2 intraplaque neovascularization assessed by contrast-enhanced ultrasound (CEUS). There are extensive microbubbles throughout the plaque.

Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-12-1529