Carotid atherosclerotic lesion echogenicity and severity on standard ultrasound is correlated with intraplaque neovascularization detected by contrast-enhanced ultrasound imaging

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- STRUCTURES, Angiogenesis < 6.
- TOPICS, Arteriosclerosis < 6.
- TOPICS, Comparative Studies < 7.
- METHODOLOGY

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- Online video.avi
Carotid atherosclerotic lesion echogenicity and severity on standard ultrasound is correlated with intraplaque neovascularization detected by contrast-enhanced ultrasound imaging

Manuscript type: original research

Advance in knowledge:

1. In agreement with the concept that more vulnerable plaques are more likely to have a greater degree of neovascularization, neovascularization within carotid atherosclerotic lesions visualized by contrast-enhanced carotid ultrasound is more pronounced in echolucent lesions (rho -0.199, p=0.001).

2. Intraplaque neovascularization on contrast-enhanced ultrasound was more pronounced in lesions with higher degree of stenosis (rho 0.157, p=0.003) and correlates with maximal lesion thickness (rho 0.233, p<0.001).

Implication for patient care:

Contrast-enhanced ultrasound may be a valuable tool for further risk stratification of atherosclerotic lesions and may provide an additional non-invasive technique to monitor therapeutic interventions.
Abstract

Purpose: To correlate echogenicity and severity of atherosclerotic carotid lesions on standard ultrasound with the degree of intraplaque neovascularization on contrast-enhanced ultrasound (CEUS) imaging.

Materials and Methods: The HIPAA-complaint study was approved by the local ethics committee and all patients provided informed consent. A total of 175 patients (mean age 67±10 years, 65% male) underwent standard and contrast-enhanced carotid ultrasound. Lesion echogenicity (Class I to IV), degree of stenosis, and maximal lesion thickness were evaluated for each documented atherosclerotic lesion. The degree of intraplaque neovascularization on CEUS was categorized as absent, moderate, or extensive. Correlation of neovascularization with echogenicity, degree of stenosis, and maximal lesion thickness was made using Spearman’s rho and chi-square test for trend.

Results: In total 293 atherosclerotic lesions, echogenicity was inversely correlated with grade of intraplaque neovascularization (rho -0.199, p=0.001). More echolucent lesions had higher degree of neovascularization compared with more echogenic ones (p<0.001). The degree of stenosis was significantly correlated with grade of intraplaque neovascularization (rho 0.157, p=0.003). Lesions with higher degree of stenosis had higher grade of neovascularization (p=0.008), and maximal lesion thickness increased with the grade of neovascularization (p<0.001) and was significantly correlated with grade of neovascularization (rho 0.233, p<0.001).

Conclusions: Neovascularization visualized by CEUS correlates with lesion severity and with morphological features of plaque instability, contributing to the concept that more vulnerable plaques are more likely to have a greater degree of neovascularization. Therefore, CEUS may be a valuable tool for further risk
stratification of echolucent atherosclerotic lesions and carotid stenosis of different degrees.

**Introduction**

Current risk stratification of carotid atherosclerotic lesions is based on the evaluation of degree of stenosis and plaque morphology using standard Duplex ultrasound imaging. Several studies have shown that patients with high degree stenosis either symptomatic or asymptomatic may benefit from carotid endarterectomy. Moreover, echolucent plaques on B-mode ultrasound are known to correspond to histological features of plaque instability. They are prone to rupture based on an increased lipid content, macrophage density as well as intraplaque hemorrhage. Echolucent plaques are also associated with higher risk for future ischemic stroke and subjects with echolucent plaques have an independently increased risk of cardiac events.

Vasa vasorum derived neovascularization is also known to be an important feature in plaque development and vulnerability triggered by inflammation and hemorrhage. The progression of plaques is associated with angiogenesis (microvessel formation) within the plaque, and eventually, these microvessels contribute to the instability of the plaque. As recently shown, contrast-enhanced ultrasound (CEUS) provides direct visualization of the carotid plaque neovascularization, using the fact that contrast agents microbubbles are ideal intravascular tracers, thus permitting a noninvasive assessment of the dynamic spatial and temporal heterogeneity of the microvasculature within the plaque. The degree of neovascularization in atherosclerotic carotid lesions detected by CEUS is well correlated with the histological density of neovessels. Furthermore, different groups found that intraplaque neovascularization in the carotid arteries detected with
CEUS was more pronounced in symptomatic patients with a history of cerebrovascular or cardiac events.\(^{(22-24)}\) The purpose of this study was to correlate echogenicity and severity of atherosclerotic carotid lesions on standard ultrasound with the degree of intraplaque neovascularization on CEUS imaging.

**Materials and Methods**

**Patients**

The HIPAA-compliant study was approved by the responsible local ethics committee (Rush University Medical Center, IRB #2, ORA# 01062001) and written informed consent was obtained from each patient. Between January 2004 and September 2008, 175 consecutive subjects with at least one atherosclerotic carotid plaque on B-mode ultrasound were enrolled. The patients underwent standard carotid ultrasound investigation followed by CEUS examination of the carotid arteries of both sides. Indications for carotid ultrasound were as follow: 4\% (7/175) TIA/Stroke within 6 months, 20\% (35/175) carotid bruit, 10\% (18/175) follow-up control of known carotid stenosis, 59\% (103/175) cardiovascular risk assessment (high cardiovascular risk profile, preoperative) or unspecific neurological symptoms (e.g. vertigo, syncope), 7\% (12/175) other indication. Exclusion criteria were known allergies to albumin, or to ultrasound contrast media. From the 175 patients enrolled in this study, 111 patients were also included in a previously published study.\(^{(24)}\)

**Ultrasound Investigations**

Carotid standard ultrasound was performed using a GE VIVID 7, (GE Healthcare, Chalfont St.Giles, United Kingdom) or ATL HDI 5000 (Philips, Best, Netherlands) ultrasound system. The examinations were performed by a trained vascular technologist using a standard imaging protocol. Both left and right carotid arteries
were examined in a supine position with the head supported at a 45° angle turned to the contralateral side. The examination consisted of B-mode ultrasound imaging, color Doppler ultrasound and pulsed Doppler spectral analysis of the common carotid artery, the extracranial segments of the internal carotid artery, and the external carotid artery. Upon completion of the non-contrast portion of the examination, ultrasound contrast agents were infused according to a protocol previously described. (20, 24)

The equipment settings for both ultrasound systems and for contrast imaging were performed as follows: the mechanical index was reduced to the lowest possible level, the focus was set below the region of interest; overall gain, time gain compensation and compression were maximized and provided as “pre sets,” however, based on the discretion of the sonographer, adjustments were made to provide highest contrast effect for each study. Specifically, for each machine the following settings were implemented: ATL HDI 5000 system, 7-4 linear array vascular probe with General Imaging harmonic software, mechanical index was set at 0.06-0.1; GE VIVID 7 Dimension system, 7L probe with harmonic software, and mechanical index was adjusted to 0.18-0.20 for maximum contrast effect.

The contrast-enhanced ultrasound studies were performed using perflutren protein type-A microspheres (Optison, GE Healthcare, Buckinghamshire, UK) or perflutren lipid microspheres (Definity, Bristol-Myers Squibb Medical Imaging, Billerica, MA) ultrasound contrast agents. The ultrasound contrast agent Optison and Definity, supplied as 3ml and 1.5ml vials were diluted with 7ml and 8.5ml of 0.9% saline, respectively, resulting in a total of 10 ml of infusate. The contrast agent was injected via a peripheral vein as a bolus of 2 ml followed by a saline bolus of 2-3 ml. The bolus was repeated when required and for the contralateral side after an interval of 1 minute. The appearance of the contrast effect was observed within the carotid lumen.
within 10 to 30 seconds following the injection sequence. Imaging after contrast injection was performed during 3 minutes at each side at the middle and distal part of the common carotid artery, the carotid bulb, and the proximal part of the internal carotid artery. The standard and contrast-enhanced ultrasound studies were recorded on VHS videotape and/or stored digitally for offline further analysis and quantification by the principle investigator (DS) with 10 years of experience of vascular ultrasound readings.

**Ultrasound Imaging Analysis**

Based on B-mode ultrasound images, the presence of atherosclerotic plaque was considered according to the Mannheim consensus as focal structures encroaching into the arterial lumen (at least 0.5 mm or 50% of the surrounding intima-media complex or an absolute thickness of the intima-media-layer greater than 1.5mm).(25) The degree of stenosis of the internal carotid artery was graded according to current guidelines as < 50%, 50-69%, or ≥ 70% stenosis based on spectral Doppler velocities.(26) The maximal thickness of the lesion was measured using a longitudinal image from the media-adventitia to the intima-lumen boundaries on B-mode ultrasound, and was assessed as continuous variable and by quartiles. Lesion echogenicity was classified according to well established criteria (27) as uniformly echolucent (Class I), predominantly echolucent (Class II), predominantly echogenic (Class III), uniformly echogenic or extensively calcified (Class IV).

Following the infusion of ultrasound contrast agent, the lumen of carotid artery was enhanced resulting in enhanced plaque luminal morphology visualization. Using a low MI setting, and the use of contrast-enhanced, harmonic software, carotid plaques and the intima-media complex appeared hypoechoic with the adventitial layer bright echogenic. The presence of blood flow “activity” was identified based on the dynamic
movement of the echogenic reflectors (microspheres) observed within the intraplaque microvessels. Fixed echogenic signals were considered to be tissue acoustic reflectors. Intraplaque neovascularization (contrast-agent enhancement) was categorized using a modified grading scale previously published:(20, 24) Grade 1 was used to indicate no appearance of moving bubbles within the plaque or microspheres only confined to the adjacent adventitial layer [Figure 1], Grade 2 considered moderate visible appearance of moving bubbles within the plaque at the adventitial side or plaque shoulder, and Grade 3 was reserved for images with extensive intraplaque neovascularization with clear visible appearance of bubbles moving to the plaque core [Figure 2].

For further analysis the highest grade of intraplaque neovascularization of each side with the corresponding lesion echogenicity, thickness and stenosis severity was applied.

Patient Characteristics

The patient’s demographics including cardiovascular risk factors, medical history, and medication at the time of carotid duplex ultrasound examination were retrospectively recorded reviewing the charts by co-workers who were blinded for the ultrasound findings of the patients. From the 175 subjects included in this analysis, 124 subjects had completed clinical data. The clinical characteristics of the study population are presented in Table 1.

Statistical Analysis

Statistical analysis was performed using SPSS/PC (Version 17.0 SPSS Inc., Chicago, Illinois). Correlation of grades of neovascularisation with echogenicity as well as with degree of stenosis and quartiles of maximal lesion thickness was made.
using Spearman’s rho and chi-square test for trend. Comparison of maximal lesion thickness between the different grades of intraplaque neovascularization was made using analysis of variance (ANOVA) for independent samples. A two-sided 𝑃 value of less than .05 was considered significant. For additional analysis of the data restricted to a single lesion per patient, the more severe lesion, based on degree of stenosis and maximal lesion thickness of each patient was studied.

## Results

### Lesion Characteristics

Among the enrolled 175 subjects, bilateral atherosclerotic carotid lesions were detected in 118 subjects. Therefore, a total of 293 lesions were documented and used for further analysis. The lesion’s characteristics are listed in Table 2. The majority of the lesions led to a < 50% stenosis. Only 6% showed a degree of stenosis ≥ 70%. Lesions were evenly distributed among different echogenicity classes.

No contrast enhancement within the atherosclerotic lesion (grade 1) was documented in 61%, moderate intraplaque neovascularization (grade 2) in 33%, and extensive plaque neovascularization (grade 3) in 6% of all lesions.

### Correlation of Lesion Echogenicity and Neovascularization

There was a negative correlation between echogenicity and grade of intraplaque neovascularization (rho -0.199, 𝑃<0.001) [Table 3]. More echolucent lesions had significantly higher degree of neovascularization compared with more echogenic ones (𝑃<0.001) [Figure 3]. The prevalence of grade 2 and grade 3 neovascularization in different classes of lesions echogenicity was 30% (9/30) and 23% (7/30) in class I, 38% (38/99) and 6% (6/99) in class II, 35% (39/111) and 5% (5/111) in class III, and 19% (10/53) and 2% (1/53) in class IV, respectively. When lesions were categorized
as echolucent (class I and II) or echogenic (class III and IV), a positive association between echolucency and more intense neovascularization was still present (p=0.02).

**Correlation of Lesion Severity and Neovascularization**

Degree of stenosis was significantly correlated with grade of intraplaque neovascularization (rho 0.157, p=0.003) [Table 3]. Lesions with higher degree of stenosis had significantly higher grade of neovascularization (p=0.008) [Figure 3]. The prevalence of grade 2 and grade 3 neovascularization in plaques determining a stenosis < 50% was 30% (68/226) and 5% (11/226), in stenosis 50% to 69% prevalence was 45% (22/49) and 8% (4/49), and in stenosis ≥ 70% prevalence was 33% (6/18) and 22% (4/18), respectively. Maximal lesion thickness increased with the grade of neovascularization (grade 1: 2.5±0.9 mm, grade 2: 2.8±0.9 mm, and grade 3: 3.6±0.9 mm; p<0.001). Maximal lesion thickness and quartiles of maximal thickness were significantly correlated with grade of neovascularization (rho 0.233, p<0.001, and 0.248, p<0.001, respectively). Higher quartiles of maximal lesion thickness had significantly higher grade of neovascularization (p<0.001) [Figure 3]. The prevalence of grade 2 and grade 3 neovascularization in the first quartile of maximal thickness (<2 mm) was 20% (14/70) and 3% (2/70), in the second quartile (2.0 to 2.4 mm) prevalence was 36% (27/75) and 0% (0/75), in the third quartile (2.5 to 3.2 mm) prevalence was 44% (32/73) and 7% (5/73), and in the last quartile (>3.2 mm) prevalence was 32% (23/73) and 16% (12/73), respectively.

**Analysis restricted to a single lesion per patient**

As shown in Table 3, correlations of lesion echogenicity and severity with intraplaque neovascularization restricted to a single carotid lesion per patient (175 lesions) were
similar compared with the analysis of all 293 lesions. In these 175 lesions, more echolucent lesions compared with more echogenic ones, lesions with higher degree of stenosis, and higher quartiles of maximal lesion thickness, had also significantly higher degree of neovascularization (p=0.003, p=0.017, and p=0.005, respectively).

Discussion

In this prospective observational study we demonstrated that prevalence and degree of neovascularization within 293 carotid atherosclerotic lesions detected by CEUS imaging was higher in more echolucent lesions and in lesions of higher severity assessed by degree of stenosis and maximal lesion thickness. The relationship between lesion density and contrast enhancement, as well as between the extent of atherosclerotic lesions and degree of neovascularization on CEUS is in agreement with the pathophysiologic concept that intimal angiogenesis is associated with more rapidly progressive and unstable vascular disease.(28)

Echolucency on standard carotid B-mode ultrasound, indicating plaques with histological features of vulnerability as lipid-rich core,(29) high macrophage density and intraplaque hemorrhage,(30) is a well known marker of lesions with higher risk for future ischemic stroke (8-10) and coronary events in patients with stable coronary disease.(13) CEUS imaging has emerged as a non-invasive tool to further stratify the risk of carotid lesions by visualizing intraplaque neovascularization.(17, 20, 21, 24) Histopathologic data in humans confirm that plaque neovascularization is an almost ubiquitous feature of atherosclerotic disease, increasing with lesion progression,(16) and symptoms.(15) These microvessels (angiogenesis) within the atherosclerotic lesions arise mostly from the adventitial vasa vasorum, stimulated by plaque hypoxia, reactive oxygen species, hypoxia-inducible factor signaling and inflammation.(31) Microvessel density is greatest in lesions with marked macrophage infiltration and
thin-cap atheroma,(32) as well as with lipid-rich lesions,(33) all features of a so-called vulnerable plaque.(2, 34) Finally, initiated and triggered by vascular cell leakage, inflammatory cell recruitment, and intraplaque hemorrhage, this ectopic neovascularization may leads to plaque rupture and consequently to cardio- and cerebrovascular events.(2, 32, 35)

Our findings are in accordance with previous reports of smaller studies comparing carotid plaque echolucency on B-mode imaging with quantitative analysis of intraplaque neovascularization on CEUS imaging (21-23) and with plaque inflammation (36) and lipid contents using also other imaging techniques as positron emission tomography (37) or MRI. (38) However, our results extended these studies to a larger number of atherosclerotic carotid lesions. Coli et al. studied 32 patients with 52 carotid plaques by standard and CEUS imaging.(21) Using a similar visual based grading system for plaque echogenicity and neovascularization, more echolucent plaques had a significantly higher degree of contrast-agent enhancement compared with more echogenic ones (p=0.001). A subgroup of 17 patients underwent carotid endarterectomy and quantitative analysis of vasa vasorum density within each histological sample was performed. Interestingly, intraplaque neovascularization on CEUS imaging did, but plaque echolucency did not correlate with histological microvessel density, suggesting that CEUS is a more specific imaging modality to identify highly vascularized and inflamed vulnerable lesions than standard ultrasound imaging alone. Similarly, Xiong et al. investigated 133 carotid plaques among 35 symptomatic and 69 asymptomatic patients with standard and CEUS imaging.(22) The percentage of hypoechoogenic, soft plaques on B-mode imaging and plaque enhancement after injection of contrast agent in the symptomatic patients was significantly greater than that in the asymptomatic patients (74% versus 38%, p=0.001, and 80% versus 30%, p<0.001, respectively). Using a visual based
semiquantitative analysis and quantitative analysis measured by video intensity of contrast enhancement within the atherosclerotic lesion, the authors reported a significantly (p<0.05) higher proportion of contrast enhancement in soft plaques compared with the other three types of plaques (hard, calcified, and mixed plaque). Furthermore, in line with the concept that angiogenesis within the atherosclerotic lesions is an important feature of plaque progression mainly driven by hypoxia (31, 39) caused by arterial wall thickening and inflammation, our results indicate greater neovascularization in more stenotic lesions and in lesions with higher thickness. However, in contrast to our results, in both aforementioned studies no significant association between degree of stenosis and prevalence of extensive contrast-agent enhancement in lesions (p=0.56),(21) as well as between plaque thickness and enhanced intensity in the plaque was found (p=0.26).(22) This discrepancy may be explained by the greater amount of atherosclerotic lesions investigated in our study, by differences in baseline characteristics of the patients population, and by differences in lesion characteristics such as a lower portion of lesions with ≥70% stenosis and less calcified plaques compared to other studies.

Cross-sectional studies of carotid endarterectomy specimens demonstrated that intraplaque angiogenesis can be reduced by statin therapy.(40) Anti-atherosclerotic therapies have shown to be rapidly effective in increasing echogenicity on standard ultrasound imaging of vulnerable plaques without regression of plaque size.(38, 41, 42) Therefore, quantification of plaque neovascularization by CEUS imaging may be an additional non-invasive technique to monitor therapeutic interventions. In patients with echolucent lesions or lesions with <70% luminal stenosis which are not subjected to carotid endarterectomy, CEUS imaging may identify highly vascularized lesions with potentially higher risk for cerebrovascular events. These patients should probably have more aggressive therapy for cardiovascular risk factors (e.g. higher
dose of statin therapy), and future studies are needed to evaluate whether these patients may benefit from prophylactic carotid endarterectomy.

Several limitations should be considered in interpreting our results. First, we used a well established semi-quantitative visual approach which has been previously published to quantify neovascularization on CEUS imaging,(20, 21, 24) and to classify echogenicity on standard ultrasound imaging of carotid lesions.(27) Intraobserver and interobserver agreement for grading intraplaque neovascularization on CEUS imaging and for grading plaque morphology on B-mode images has been previously published. (24, 27) Briefly, for intraplaque neovascularization, intraobserver agreement was substantial (kappa coefficient 0.63), and interobserver agreement was moderate (kappa coefficient 0.54). For both, intra- and interobserver agreement for grading lesion echogenicity (4-category classification) using digital images were moderate (pooled kappa coefficient 0.53 ± 0.06, and 0.52 ± 0.05, respectively). In future applications, it is anticipated that the use of computer-assisted quantitative analyses of intraplaque neovascularization,(22, 43), and echogenicity,(44) as well as 3D volume data sets will provide additional value.(18) Second, in hyperechoic and calcified plaques with acoustic shadowing, the detection of contrast-agent microbubbles can be restricted and may interfere with our results. However, it is known that fibrocalcific plaques in diabetic patients who normally have extensive vascularization of atherosclerotic lesions,(45) are no longer vascularized, suggesting that microvessel involution may be a marker for plaque stabilization.(32) In accordance with this concept, we found less extensive neovascularization in more echogenic and therefore most likely more fibrocalcific lesions. Furthermore, only a single vessel segment with each contrast injection can be studied. Third, as our analysis was based on 293 carotid lesions from 175 patients
without any consideration of clustering, it may incorrectly treat the lesions as independent. However, additional analysis restricted the data to a single lesion per patient have shown similar results, supporting a negligible clustering effect in our analysis.

Fourth, this is an observational study with analysis of carotid lesions using and comparing different ultrasound imaging techniques, but without analysis of clinical endpoints. In previous studies, including a study from our group, intraplaque neovascularization detected by CEUS imaging was already linked to the patient’s symptoms. Different from the present study we included in the previous study patients with and without carotid atherosclerotic lesions, and correlated degree of vessel wall vasa vasorum and intraplaque neovascularization on CEUS with clinical data, namely cardiovascular disease and history of cardiovascular events. We did not assess and correlate intraplaque neovascularization with plaque echogenicity, degree of stenosis, and maximal lesion thickness in the previous study. Besides, we used a different, less sophisticated semi-quantitative visual approach to quantify neovascularization on CEUS imaging compared to the present study. Furthermore, given the low proportion of patients having severe stenosis in the present study, our results might not be applicable to a population undergoing carotid ultrasound in a different clinical setting. Therefore, prospective clinical trials are needed to determine the potential impact of CEUS imaging for stratifying the risk of carotid atherosclerotic lesions to prevent future cerebrovascular events.

These results substantiate and extend previous findings of the association between intraplaque neovascularization and lesion echogenicity to a greater patient collective of 175 subjects with overall 293 carotid lesions. Our results support the hypothesis that neovascularization visualized by CEUS is correlated with morphological features
of plaque instability and with lesion severity. The positive relationship between lesion echolucency, degree of stenosis, maximal lesion thickness, and contrast-agent enhancement is in agreement with the concept that more advanced atherosclerotic plaques prone for rupture, are more likely to have a greater degree of neovascularization. Therefore, CEUS may be a valuable tool for further risk stratification of echolucent atherosclerotic lesions and carotid stenosis of different degrees.
References


<table>
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<th>Table 1. Clinical characteristics of the study population</th>
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<td>Patient population (n=175)</td>
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<td>Age (y)*</td>
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<tr>
<td>In men†</td>
</tr>
<tr>
<td>In women†</td>
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<td>Male sex</td>
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<td>Clinical history</td>
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</tr>
<tr>
<td>TIA</td>
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<tr>
<td>Stroke</td>
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<td>PAD</td>
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Unless otherwise indicated, data are numbers of patients and data in parentheses are percentages. *Data are means ± standard deviations. †Date are means (range). ‡Neurological symptoms (TIA/stroke) within 6 months.

BMI = body mass index; CAD = coronary artery disease; HDL = high density lipoprotein; LDL = low density lipoprotein; PAD = peripheral artery disease; TIA = transient ischemic attack.
Table 2. Sonographic characteristics of the atherosclerotic carotid lesions

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<tr>
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<td><strong>Severity</strong></td>
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<tr>
<td>Stenosis &lt; 50%</td>
<td>226 (77)</td>
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<tr>
<td>PSV (cm/s)*</td>
<td>90±22 (32 – 161)</td>
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<tr>
<td>Stenosis 50-69%</td>
<td>49 (17)</td>
</tr>
<tr>
<td>PSV (cm/s)*</td>
<td>166±35 (124 – 245)</td>
</tr>
<tr>
<td>Stenosis ≥ 70%</td>
<td>18 (6)</td>
</tr>
<tr>
<td>PSV (cm/s)*</td>
<td>315±70 (233 – 464)</td>
</tr>
<tr>
<td>Maximal lesion thickness (mm)*</td>
<td>2.7±0.9 (1.3 to 7.9)</td>
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<td><strong>Echogenicity</strong></td>
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<tr>
<td>Class I</td>
<td>30 (10)</td>
</tr>
<tr>
<td>Class II</td>
<td>99 (34)</td>
</tr>
<tr>
<td>Class III</td>
<td>111 (38)</td>
</tr>
<tr>
<td>Class IV</td>
<td>53 (18)</td>
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<tr>
<td><strong>Neovascularization</strong></td>
<td></td>
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<tr>
<td>Grade 1</td>
<td>178 (61)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>96 (33)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>19 (6)</td>
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Unless otherwise indicated, data are numbers of patients and data in parentheses are percentages. *Data are means ± standard deviations and data in parentheses indicate the range. PSV = peak systolic velocity on Doppler ultrasound
Table 3. Correlations of intraplaque neovascularization with carotid lesion echogenicity and severity

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Correlations with intraplaque neovascularization (Grade 1 - 3) on CEUS, Spearman’s rho, p-value</th>
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<td>All lesions (n=293)</td>
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<td>One single lesion per patient (n=175)</td>
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<td>Echogenicity (Class I – IV)</td>
<td>-0.199, p&lt;0.001</td>
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<tr>
<td></td>
<td>-0.245, p=0.001</td>
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<tr>
<td>Degree of stenosis (&lt;50%, 50-69%, ≥ 70%)</td>
<td>0.157, p=0.003</td>
</tr>
<tr>
<td></td>
<td>0.233, p=0.002</td>
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<tr>
<td>Maximal lesion thickness (mm)</td>
<td>0.233, p&lt;0.001</td>
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<tr>
<td></td>
<td>0.221, p=0.003</td>
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</table>

CEUS = contrast-enhanced ultrasound
**Figure 1.** Hyperechogenic carotid lesion on standard ultrasound without intraplaque neovascularization on contrast-enhanced ultrasound imaging.

This carotid ultrasound examination was obtained from a 73-year old asymptomatic woman with carotid bruit and previous documented carotid stenosis for follow-up control. Panel A: Uniformly echogenic (class IV) lesion on B-mode ultrasound imaging at the origin of the internal carotid artery. Panel B: Corresponding presentation on contrast-enhanced ultrasound imaging without microbubbles within the lesion (circled region in red) (grade 1).

**Figure 2.** Hypoechogenic carotid lesion on standard ultrasound with intraplaque neovascularization on contrast-enhanced ultrasound imaging.

This 71-year old women presented with symptomatic cerebrovascular disease (TIA). Panel A: Predominantly echolucent (Class II) lesion on B-mode ultrasound imaging at the origin of the internal carotid artery. Panel B: Corresponding presentation on contrast-enhanced ultrasound imaging. The circled region in red highlights the clear visible appearance of microbubbles moving to the plaque core (grade 3). (Also see online video)

**Figure 3.** Grade of intraplaque neovascularization on contrast-enhanced ultrasound according to lesion echogenicity, degree of stenosis, and quartiles of maximal lesion thickness on standard ultrasound imaging.

Panel A: The proportion of carotid lesions with higher grade of intraplaque neovascularization is greater in more echolucent lesions ($p<0.001$ by chi-square analysis for trend). Panel B: The proportion of carotid lesions with higher grade of intraplaque neovascularization is greater in lesions with higher degree of stenosis ($p=0.008$ by chi-square analysis for trend). Panel C: The proportion of carotid lesions...
with higher grade of intraplaque neovascularization is greater in lesions of higher quartile of maximal lesion thickness (p<0.001 by chi-square analysis for trend).

**Movie caption:** Intraplaque neovascularization on contrast-enhanced ultrasound imaging. Carotid lesion at the origin of the internal carotid artery on contrast-enhanced ultrasound imaging with clear visible appearance of microbubbles moving to the plaque core (grade 3).
Figure 1A Hyperechogenic carotid lesion on standard ultrasound without intraplaque neovascularization on contrast-enhanced ultrasound imaging.

This carotid ultrasound examination was obtained from a 73-year old asymptomatic woman with carotid bruit and previous documented carotid stenosis for follow-up control. Panel A: Uniformly echogenic (class IV) lesion on B-mode ultrasound imaging at the origin of the internal carotid artery.

138x83mm (300 x 300 DPI)
**Figure 1B** Panel B: Corresponding presentation on contrast-enhanced ultrasound imaging without microbubbles within the lesion (circled region in red) (grade 1).

116x84mm (300 x 300 DPI)
Figure 2A Hypoechoic carotid lesion on standard ultrasound with intraplaque neovascularization on contrast-enhanced ultrasound imaging.

This 71-year old women presented with symptomatic cerebrovascular disease (TIA). Panel A: Predominantly echolucent (Class II) lesion on B-mode ultrasound imaging at the origin of the internal carotid artery.

126x91mm (300 x 300 DPI)
Figure 2B: Corresponding presentation on contrast-enhanced ultrasound imaging. The circled region in red highlights the clear visible appearance of microbubbles moving to the plaque core (grade 3). (Also see online video)

127x91mm (300 x 300 DPI)
Figure 3 Grade of intraplaque neovascularization on contrast-enhanced ultrasound according to lesion echogenicity, degree of stenosis, and quartiles of maximal lesion thickness on standard ultrasound imaging.

Panel A: The proportion of carotid lesions with higher grade of intraplaque neovascularization is greater in more echolucent lesions (p<0.001 by chi-square analysis for trend). Panel B: The proportion of carotid lesions with higher grade of intraplaque neovascularization is greater in lesions with higher degree of stenosis (p=0.008 by chi-square analysis for trend). Panel C: The proportion of carotid lesions with higher grade of intraplaque neovascularization is greater in lesions of higher quartile of maximal lesion thickness (p<0.001 by chi-square analysis for trend).

154x135mm (600 x 600 DPI)