



Advance ultrasound techniques for the assessment of plaque vulnerability in symptomatic and asymptomatic carotid stenosis: a multimodal ultrasound study

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Background: Advanced carotid ultrasound techniques may be useful in characterizing plaque vulnerability, but comprehensive studies are still lacking. The aim of this study was to identify factors associated with vulnerable plaques using advanced ultrasound techniques.

Methods: This is a prospective observational study of patients with >50% internal carotid stenosis (ICA). All patients underwent conventional ultrasound, superb microvascular imaging (SMI) and shear wave elastography (SWE) examinations. Plaque size, echogenicity, stiffness and intraplaque neovascularization (IPN) were assessed and compared between symptomatic and asymptomatic groups. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic performance of SWE and SMI of the vulnerable plaques.

Results: The final analysis included 123 patients (78.9% male; mean age, 66±8 years), 65 were enrolled in the symptomatic group, and 58 were enrolled in the asymptomatic group. The mean elasticity was 78.1±25.4 kPa for asymptomatic and 51.5±18.3 kPa for symptomatic plaques. Symptomatic plaques showed higher visual IPN grades on SMI than asymptomatic plaques ($P<0.001$). Multivariate regression analysis showed that plaque stiffness (PS) (OR 0.95, 95% CI, 0.919–0.974) and IPN level (OR 4.17, 95% CI, 2.008–8.664) were independently associated with symptomatic plaques. The combination of the two factors had a preferable accuracy to discriminate symptomatic plaques (AUC 0.89, 95% CI, 0.827–0.944).

Conclusions: Advanced carotid ultrasound techniques can identify plaque characteristics that are associated with ischemic events and may be potentially indicative of plaque vulnerability. These factors may ultimately be used in the clinical management of carotid stenosis.

Keywords: Carotid plaque; vulnerability; shear wave elastography (SWE); superb microvascular imaging (SMI); stroke

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Introduction

Stroke is a public health problem and is the leading cause of death and disability worldwide (1). Current guidelines for stroke prevention in patients with carotid plaques are based on the severity of lumen narrowing, and this factor is

important for decision making to prevent stroke occurrence (2,3). Mounting evidence shows that vulnerable plaques are more likely to lead to ischemic events, independent of the severity of carotid stenosis (4,5). Therefore, risk stratification of patients with asymptomatic carotid

stenosis is necessary to improve target patient selection for interventional therapies, and carotid plaque vulnerability assessment may help fulfill this goal.

Atherosclerotic plaques associated with a high likelihood of neurological event occurrence and those that progress rapidly should be considered vulnerable plaques (6). Based on histopathological findings, several key plaque composition characteristics such as a thin fibrous cap, a large lipid rich necrotic core, intraplaque hemorrhage (IPH), inflammatory and intraplaque neovascularization (IPN), are associated with vulnerable plaques. New imaging techniques that can identify vulnerable carotid plaques *in vivo* are therefore needed for more accurate targeting of prophylactic therapy and preventing the occurrence of stroke.

Carotid ultrasound is a noninvasive and widely used imaging approach for carotid plaque (atherosclerosis) screening. Conventional ultrasound techniques have enabled routine characterization of carotid plaque features (e.g., in plaque area, echogenicity and surface morphology) (7). Shear wave elastography (SWE) is an emerging ultrasound-based technique that enables quantitative and noninvasive assessment of tissue stiffness (8). Elastography ultrasound has become increasingly important for differentiation of the characteristics of solid tumors, especially in the breast, liver, and thyroid (9). SWE provides additional information related to plaque characteristics such as the presence of a large lipid core and IPH, which may indicate plaque vulnerability (10,11). However, currently, most of the studies on the assessment of carotid plaque vulnerability by elastography have been performed on small samples of patients. IPN, a well-known marker in plaque destabilization, can be assessed by contrast-enhanced ultrasound (CEUS) as contrast-agent enhancement in the plaque (12). However, CEUS is a minimally invasive examination that requires an intravenous injection of a contrast-agent with a related risk that limits its use in clinical practice. Superb microvascular imaging (SMI) is a novel ultrasound technique devised for overcoming the limitations of conventional Doppler ultrasound, which can successfully visualize the microvascular blood flow signals without the use of contrast agents (13).

Our postulate is that plaque composition characteristics, such as IPN and plaque stiffness (PS), will be significantly difference in plaques that cause ipsilateral neurological symptoms compared to asymptomatic plaques. Accordingly, we hypothesize that IPN detected by SMI without using contrast agent is a surrogate mark of plaque vulnerability and that SWE provides more additional information about

plaque vulnerability, and stroke risk.

We present the study in accordance with the STARD reporting checklist (available at <http://dx.doi.org/10.21037/cdt-20-876>).

Methods

Our present study was approved by the Institutional Review Board of Beijing Tiantan Hospital (IRB No. KY2019-113-01). All participants gave written informed consent before SWE and SMI examinations. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Study population

In this prospective observational study, patients with >50% internal carotid artery stenosis, as determined by carotid duplex ultrasound, were consecutively included at Beijing Tiantan Hospital, from December 2018 to February 2020. This population was separated into two groups: symptomatic with an ipsilateral carotid-related ischemic event (amaurosis fugax, transient ischemic attack, or stroke) within the past 30 days, and asymptomatic without any ischemic events in the corresponding carotid territory within the past 6 months. This classification based on ischemic events was used as a surrogate marker of plaque vulnerability. The exclusion criteria were patients who received previous radiation therapy in the neck, patients diagnosed with nonatherosclerotic carotid artery stenosis, patients with >70% ipsilateral intracranial artery stenosis confirmed by CTA or MRA, patients with severe disturbance of consciousness, and those in whom plaque visualization was difficult because of an acoustic shadow due to severe calcification. A neurologist who was blinded to the ultrasound findings recorded clinical data from all participants, including age, sex, body mass index (BMI), risk factors for atherosclerosis, history of cardiovascular disease, use of medications, and ischemic events. All participants underwent conventional ultrasound, SMI, and SWE of the carotid arteries before CEA during hospitalization or at a routine outpatient follow-up visit for asymptomatic carotid stenosis.

Conventional ultrasound protocol

All imaging was performed using the same ultrasound machine (Aplo 900, Canon Medical Systems Corporation, Japan), and a 14L5 (frequency range, 5–14 MHz) linear

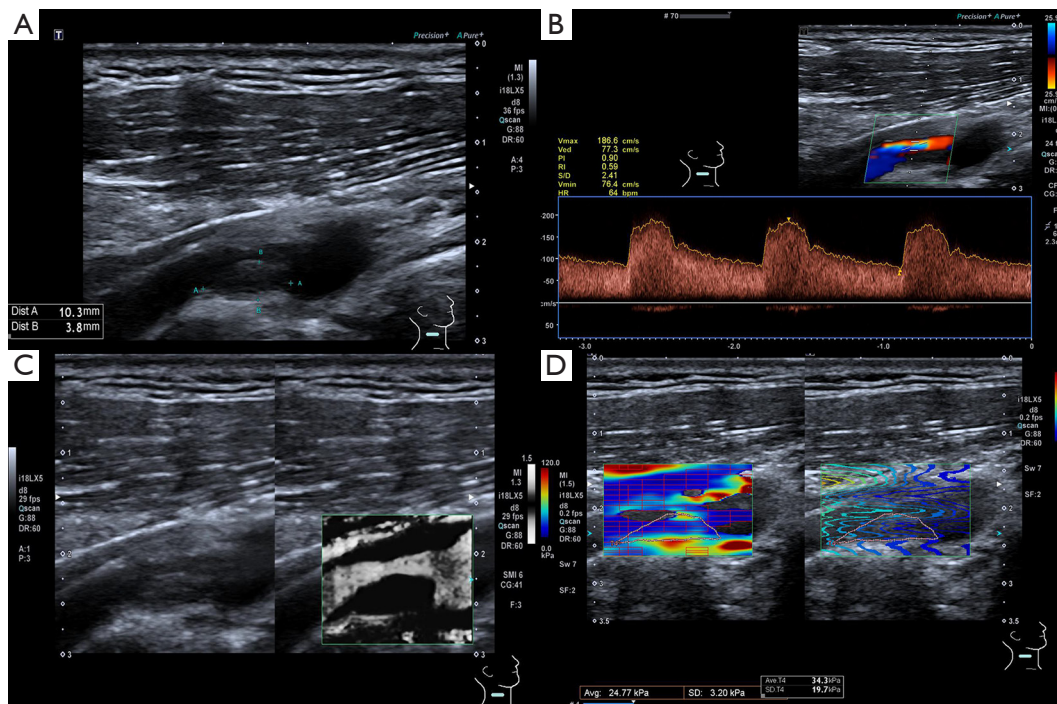


Figure 1 Example of a multimodal ultrasound study. (A) Grayscale ultrasound showed a predominantly hypoechoic plaque located at posterior wall of right proximal internal carotid artery. (B) Pulsed wave Doppler ultrasound showed a strong increase of peak systolic velocity caused by a hemodynamically significant stenosis. (C) SMI in longitudinal view confirmed the high-grade stenosis; no ulcerations were seen along the border of the plaque; no IMVF is visible within the plaque. (D) SWE showed the Young's modulus of the plaque was 34.3 kPa, quality control map confirmed that the sampling was done correctly. IMVF, intraplaque microvascular flow; SMI, superb microvascular imaging; SWE, shear wave elastography.

array probe was used for the conventional ultrasound, SMI and SWE examinations (*Figure 1*). With the patient in the supine position, the bilateral carotid arteries (including the common carotid artery, carotid bifurcation, and internal carotid artery) were scanned in longitudinal and transverse sections in standard carotid ultrasound. All images were independently assessed by two vascular sonographers with five years' experience who were blinded to clinical outcomes.

The degree of internal carotid artery stenosis was graded as follows: 50–69% and 70–99% determined based on peak-systolic and end-diastolic velocities according to the ultrasound consensus criteria (14). A carotid plaque was defined as an intima-media thickness greater than 1.5 mm or a focal wall thickening of at least 50% greater than the surrounding vessel wall (15). The maximal internal carotid plaque thickness (MICPT), plaque size (area), presence of calcification (yes or no) and plaque echogenicity were collected. Plaque features were assessed in longitudinal

sections, and on an image showing the thickest plaque, the intimal-medial wall thickness was measured as the MICPT. In the same image, a plaque was manually delineated and recorded as the plaque size. Plaque echogenicity was classified visually into four groups as follows (16): type 1, uniformly hypoechoic; type 2, predominantly hypoechoic; type 3, predominantly hyperechoic; and type 4, uniformly hyperechoic.

SMI protocol

After the conventional ultrasound examination, the setting of the ultrasound scanner was switched to monochrome SMI mode to show a double display of the plaque of interest in grayscale mode and monochrome SMI mode side-by-side. After starting the proprietary monochrome SMI mode, the SMI specific region of interest box was placed around the whole plaque/stenosis. Other SMI technical parameters were modified as follows: mechanical index of 1.5, frame

rate of 50–60 fps, dynamic range of 55–60 dB, and SMI velocity range of 1.0–2.0 cm/s. Plaques were first observed in the transverse section and then in the longitudinal section for 2 minutes, and the dynamic video images were stored on the device hard disk. Moving enhancements were defined as intraplaque microvascular flow (IMVF), and static enhancements (considered to be tissue acoustic reflector) were excluded. IMVF levels were classified on a visual scale according to the previous study proposed by Mahtab Zamani as follows: grade 0, no IMVF or IMVF confined to the adjacent adventitia; grade 1, moving IMVF confined to the adventitial side; grade 2, moving IMVF at the plaque shoulder; grade 3, IMVF moving to the plaque core; and grade 4, extensive IMVF (17). SMI assessments were carried out in forty plaques from grade 0 to grade 4 IMVF on 2 occasions, which were >2 weeks apart to obtain the intra- and interobserver variability using κ statistics.

SWE protocol

SWE was performed after the SMI examination with the same ultrasound unit; to obtain a twin-view display of the plaque of interest on the elastographic map and quality control map, inbuilt elasticity software was used. The ultrasound scanner both generated and estimated the propagation speed of the resulting shear waves in order to calculate the Young's modulus (YM, kPa), based on the formula $YM = \rho c^2$, where YM is tissue elasticity, ρ is tissue density and c is shear wave velocity. The scanner SWE settings were optimized based on initial findings as follows: resolution =3; smoothing =3; FR control =3; and focus =75%. The shear wave scale was set at a range from 0 kPa to 120 kPa. The SWE specific region of interest box was adjusted to include the entire carotid plaque (typically 1 cm wide \times 2 cm long) (18). With the carotid plaque in a maximal longitudinal view, the SWE procedure was started. The operator placed the probe on the skin without pressure in order to minimize pressure artifact and kept the imaging stable for approximately 3 s to allow elasticity measurements. This imaging system displays the elasticity in real time by means of a colorimetric map within the elastographic rectangular box. The measurement was manually delineated around the entire plaque. Then, the quantitative elasticity of the plaque was available. The imaging system also generates a quality control map that represents the propagation of the shear wave as wavefront lines. When the propagation lines are parallel to each other, the measurement may be more reliable and accurate;

otherwise, if the propagation lines are twisted or missing, repeated measurements may be needed. SWE assessments were carried out in forty plaques to obtain the interobserver variability using the intraclass correlation coefficient (ICC).

Conventional ultrasound images, SMI and SWE frames and cine loops were stored in the local picture archive and communication system (PACS).

Statistical analysis

All statistical analyses were performed using SPSS 22.0 software (IBM Corporation, New York, USA). Continuous variables are expressed as the means and standard deviations, while categorical variables are expressed as frequencies and percentages. Univariate analysis was performed using a t -test for continuous variables and a χ^2 test for categorical variables. Multivariate analysis was performed using binary logistic regression models with plaques associated with ischemic symptoms in patients as the dependent variable and plaque characteristics (area, echogenicity, IPN grades, and PS) as independent variables. Variables with $P < 0.10$ in the univariate analysis were included in the models. Receiver operating characteristic (ROC) curves and the area under the curve (AUC) were used to evaluate the clinical discrimination potential. Delong test was used to compare different AUC. A P value < 0.05 was considered statistically significant.

Results

Patient characteristics

We included 169 patients with >50% internal carotid artery stenosis during the study period: 9 patients with severe stroke, 4 patients who received neck radiotherapy, 20 patients without MRA or CTA above the aortic arch, 4 patients diagnosed with carotid artery dissection, 4 patients diagnosed with Takayasu's arteritis, and 5 patients with poor ultrasound imaging quality were excluded. Finally, 123 patients, including 97 men (65 ± 8 years) and 26 women (67 ± 8 years) who had complete conventional ultrasound, SMI and SWE data were eligible; 65 of these participants had symptomatic plaques, whereas the rest had asymptomatic plaques.

The baseline characteristics of the included patients are listed in *Table 1*. Symptomatic and asymptomatic groups did not significantly differ with respect to demographics or medications used. Hypertension was more frequent (but

not significantly) in asymptomatic group, and dyslipidemia was more prevalent in symptomatic group ($P=0.026$). Fifty-six (45.5%) patients had moderate stenosis, and sixty-seven patients had severe stenosis. Symptomatic plaques more commonly had severe carotid stenosis, whereas asymptomatic plaques more commonly had moderate carotid stenosis ($P<0.001$).

Plaque features

The mean plaque thickness and mean plaque area were 0.38 ± 0.08 cm (0.23 – 0.58 cm) and 0.61 ± 0.21 cm² (0.21 –

1.11 cm²), respectively, with no significant differences between symptomatic and asymptomatic groups. The proportions of different plaque echogenicities including uniformly hypoechoic plaques, predominantly hypoechoic plaques, predominantly hyperechoic plaques, and uniformly hyperechoic plaques were 8.9% (11/123), 41.5% (51/123), 30.1% (37/123), and 19.5% (24/123), respectively. The percentage of calcified plaques was 55.3% (68/123), and that of noncalcified plaque was 44.7% (55/123). IMVF signals on SMI were found in 104 (84.6%) of the 123 plaques. On SMI imaging, 19 (15.4%) plaques had no IMVF signals, 38 (30.9%) plaques had IMVF signals confined to the adventitial side, 42 (34.1%) plaques had IMVF signals at the plaque shoulder, 22 (17.9%) plaques had IMVF signals moving to the plaque core, and 2 (1.6%) plaques were found to have extensive IMVF signals. Significant differences were observed between the symptomatic and asymptomatic groups with respect to the different grades of IMVF ($P<0.001$) (Figure 2A). The mean PS was 64.2 ± 25.7 kPa, ranging from 23.2 to 126.4 kPa. Symptomatic plaques (51.5 ± 18.3 kPa) were softer than asymptomatic plaques (78.5 ± 25.4 kPa) ($P<0.001$) (Figure 2B). The mean YM of plaques in the moderate stenosis (50–69%) group was 72.3 ± 25.5 kPa, and that in the severe stenosis (70–99%) group was 57.5 ± 23.8 kPa ($P<0.001$) (Figure 2C). Carotid plaque features and comparisons between symptomatic and asymptomatic groups are summarized in Table 2.

The intra- and interobserver variability for the assessment of IPN using the 5-level category with SMI was favorable, with kappa coefficients of 0.778 and 0.705, respectively, between the 2 independent sonographers. The interrater variability for the assessment of PS using SWE

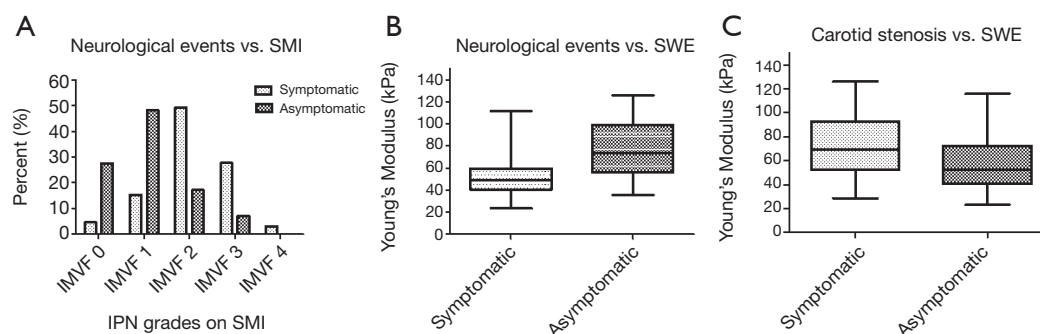


Figure 2 Graphs illustrating key findings of our study. (A) Distribution of visualization IPN grades between symptomatic and asymptomatic patients. (B) The relationship between plaque YM and neurological events, grouped into symptomatic and asymptomatic. (C) Plaque YM against the degree stenosis, grouped into either moderate (50–70%) or severe (70–99%). IPN, intraplaque neovascularization; IMVF, intraplaque microvascular flow; YM, Young's modulus; SMI, superb microvascular imaging; SWE, shear wave elastography.

Table 2 Carotid plaque features and their comparison between symptomatic and asymptomatic patients

Characteristics	Symptomatic (n=65)	Asymptomatic (n=58)	Univariate analysis P	Multivariate analysis		
				OR	CI	P
MICPT (cm)	0.38±0.07	0.39±0.09	0.344			
Plaque area (cm ²)	0.65±0.19	0.58±0.22	0.067	3.05	0.25–37.46	0.384
Calcified plaque	53.8% (n=35)	56.9% (n=33)	0.734			
Plaque echogenicity			0.156			
Uniformly hypoechoic	9.2% (n=6)	8.6% (n=5)				
Predominantly hypoechoic	49.2% (n=32)	32.8% (n=19)				
Predominantly hyperechoic	24.6% (n=16)	36.2% (n=21)				
Uniformly hyperechoic	16.9% (n=11)	22.4% (n=13)				
IPN level			<0.001	4.19	2.09–8.40	<0.001
Grade 0	4.6% (n=3)	27.6% (n=16)				
Grade 1	15.4% (n=10)	48.3% (n=28)				
Grade 2	49.2% (n=32)	17.2% (n=10)				
Grade 3	27.7% (n=18)	6.9% (n=4)				
Grade 4	3.1% (n=2)	0				
PS, kPa	51.5±18.3	78.5±25.4	<0.001	0.95	0.93–0.97	<0.001
Degree of stenosis			<0.001	5.27	1.79–15.55	0.003
50–69%	26.2%	67.2%				
70–99%	73.8%	32.8%				

OR, odds ratio; CI, confidence interval; MICPT, maximal internal carotid plaque thickness; IPN, intraplaque neovascularization; PS, plaque stiffness.

was found to have an ICC =0.911 (95% CI, 0.848–0.948) between the 2 independent sonographers.

Factors independently associated with symptomatic plaques

Among variables with a univariate $P<0.1$, dyslipidemia, the degree of stenosis, plaque area, IPN level, and PS were included in the multivariate model. The degree of stenosis (OR 5.27, 95% CI, 1.79–15.55, $P=0.003$), IPN level (OR 4.19, 95% CI, 2.09–8.40, $P<0.001$) and PS (OR 0.95, 95% CI, 0.93–0.97, $P<0.001$) were independently associated with symptomatic plaques (Table 2).

ROC curve analysis

The accuracy of differentiating symptomatic plaques from asymptomatic plaques were assessed using carotid stenosis degree, IPN level, PS and the combination of IPN level

and PS (Figure 3). The combination of the IPN level and PS yielded the best agreement to classify symptomatic plaques (AUC =0.89, 95% CI, 0.827–0.944). Delong test showed that the combination of IPN level and PS model was significantly superior to that of the other three factors alone (all $P<0.05$). For SWE, SMI, and their combination, the sensitivity was 84.5%, 80% and 76.9%, respectively, and the specificity was 70.8%, 75.9% and 87.9%, respectively, for the identification of symptomatic plaques.

SMI and SWE in moderate carotid stenosis

To explore the relationship of the SMI and SWE with symptomatic plaques in patients where additional information to carotid stenosis severity might be most useful for clinical management relating to carotid intervention, we elaborated our analyses after exclusion of patients with high-grade carotid stenosis. The subgroup with moderate

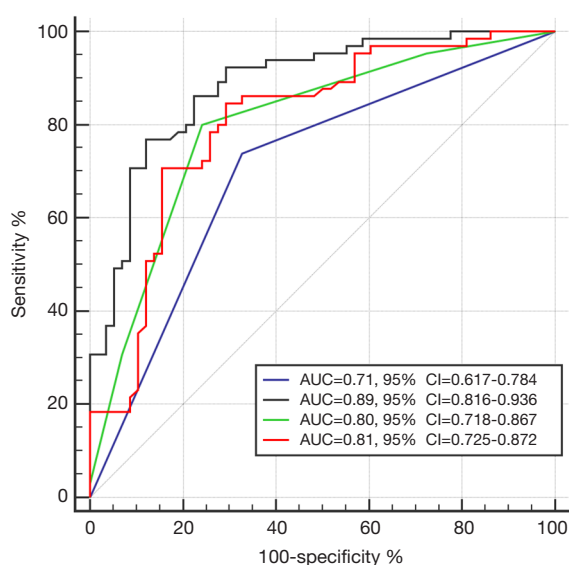


Figure 3 Receiver operating characteristic (ROC) curves. The agreement of differentiating symptomatic plaque from asymptomatic plaque was measured using carotid stenosis degree, IPN level, PS and the combination of IPN level and PS: ROC curve of carotid stenosis degree (blue line), IPN level (green line), PS (red line), and IPN level plus PS (black line). The combination of IPN level and PS (black line) yielded the best accuracy. IPN, intraplaque neovascularization; PS, plaque stiffness; AUC, area under the ROC curve; CI, confidence interval.

carotid stenosis included 56 patients, with 17 symptomatic plaques.

On univariate logistic regression analysis, hypertension, IPN level, and PS were included in the multivariate model. The IPN level (OR 3.4, 95% CI, 1.36–8.45, $P=0.009$) and PS (OR 0.95, 95% CI, 0.91–0.99, $P=0.019$) were independently associated with symptomatic plaques. On ROC analysis, the AUC of IPN level, PS, and their combination were 0.79 (95% CI, 0.66–0.92), 0.82 (95% CI, 0.71–0.95), and 0.87 (95% CI, 0.78–0.96), respectively.

Discussion

In clinical practice, the most widely accepted predictor of stroke is the degree of lumen narrowing in carotid atherosclerosis. However, among asymptomatic patients, carotid stenosis degree cannot provide sufficient evidence for identification the risk of stroke, so additional imaging characteristics for assessing plaque composition are becoming significant factors (19). Therefore, only a

comprehensive assessment of the plaque features can more precisely stratify patients of stroke risk and guide target treatment. We summarize findings of the existing studies in Table 3. No study has, on the other hand, systematically investigated the association of IPN level or PS with symptomatic plaques in such large sample size, however.

Our study confirms the clinical potential of advanced ultrasound techniques including SWE and SMI to identify high-risk carotid plaques and to differentiate symptomatic from asymptomatic plaques. Plaques with higher SMI grades were more prevalent among symptomatic plaques than among asymptomatic plaques. On SWE imaging, symptomatic plaques were significantly softer than asymptomatic plaques. Multivariate analysis revealed that IPN grades and PS were independently associated with symptomatic plaques. The combination of these two factors differentiated symptomatic plaques from asymptomatic plaques better than each factor alone. These findings suggested that the addition of advanced ultrasound techniques with conventional ultrasound improved diagnostic performance for the detection of vulnerable carotid plaques in patients with asymptomatic carotid stenosis.

The presence of newly generated small blood vessels arising from the adventitia within atherosclerotic lesions leading to IPH plays a key role in plaque destabilization and is therefore associated with neurological symptoms (24). Recently, visualization of the adventitial vasa vasorum and IPN has emerged as a new potential surrogate marker for vulnerable plaques (25). Conventional Doppler techniques filter out low-velocity signals, preventing the visualization of neovascularization. CEUS has shown preferable ability in the visualization of microvessels; however, it is a minimally invasive examination and requires intravenous injection of ultrasound contrast agents (12,26).

SMI is a novel noninvasive technique that can successfully detect microvascular blood flow signals without using ultrasound contrast agents (20). Previously published study showed good agreement between CEUS and SMI in the assessment of IPN (21). Zamani *et al.* (17) reported that plaques with higher IPN grades had higher numbers of neovessels quantified at histology ($P=0.041$, $r=0.460$). In their research, however, there was no statistically significant difference between symptomatic and asymptomatic patients with regard to the different levels of IMVF on SMI, which may be because of the relatively small sample size. In our present large-sample study, significant differences were observed between the symptomatic and asymptomatic

Table 3 Summary of previous studies on SMI and SWE of patients with carotid atherosclerosis

Article	Setting	Technique	Sample size, n	Main findings
(10)	Patients with $\geq 50\%$ carotid stenosis who underwent carotid MRI	Ultrasound NIVE	31	Ultrasound elastography is feasible in patients with carotid stenosis and can detect the presence of a lipid core with high sensitivity and moderate specificity
(11)	Patients with ischemic events who underwent carotid endarterectomy	SWE	25	The mean YM of vulnerable plaques was significantly lower than that of stable plaques (50.0 vs. 79.1 kPa; $P=0.027$). The presence of plaque hemorrhage, thrombus and increasing numbers of foam cells was also associated with a significantly lower YM
(13)	Patients with a carotid plaque (intima-media thickness ≥ 2 mm)	SMI and CEUS	27	Intraplaque enhancement was observed in 19 patients using CEUS, while intraplaque microvascular flow level signals were observed in 12 patients using SMI. A 100% specificity was recorded for SMI, while its sensitivity was 63%
(17)	Patients with $>50\%$ carotid stenosis who underwent carotid endarterectomy	SMI and CEUS	31	SMI had a positive correlation in visualization IPN with CEUS; Plaques with higher SMI grades had higher numbers of microvessels quantified at histology; Higher visual IPN counts on SMI were associated with (I) increased areas of inflammation, and (II) combined rank scores of granulation tissue, inflammation and lipids at histology
(20)	Patients with $>50\%$ carotid stenosis who underwent carotid endarterectomy	SMI	28	There was a significant correlation between the density of neovascularization in histopathologic plaques and the intraplaque microvascular flow level found by SMI. A significant difference was observed in SMI intraplaque microvascular flow level between the symptomatic and asymptomatic groups
(21)	Patients with ischemic events who had a carotid plaque	SMI and CEUS	82	Patients after 6 months atorvastatin treatment, SMI-detected intraplaque neovascularization reduced from 69.23% to 48.72%, while CEUS-detected ones reduced from 76.92% to 69.23%. The consistency between CEUS and SMI was above 0.75 at all assessments
(22)	Patients with carotid atherosclerotic plaques (luminal stenosis of 50–70%)	SMI and CEUS	125	Cox regression revealed that SMI level were the independent factor predicting ischemic stroke. There was a positive correlation between SMI grade and enhancement intensity. The AUC of SMI level predicting ischemic stroke was 0.878. The best diagnostic point was \geq level II, and its sensitivity and specificity was 86.05% and 79.27%. As the SMI grade gradually increased, the incidence of ischemic events increased gradually
(23)	Patients with carotid stenosis $>30\%$	SWE	54	Plaques associated with ischemic symptoms had significantly lower mean YM than plaques in asymptomatic patients (62 vs. 88 kPa). ROC analysis demonstrated improvements in sensitivity and specificity when percentage stenosis was combined with the YM (AUC =0.78)

NIVE, non-invasive vascular elastography; SWE, shear wave elastography; YM, Young's modulus; SMI, superb microvascular imaging; CEUS, contrast-enhanced ultrasound; IPN, intraplaque neovascularization; AUC, area under curve; ROC, receiver operating characteristic.

patients with respect to the different grades of IMVF using SMI ($P<0.001$). Only three (15.8%) among 19 patients with non-neovascularized plaques had neurological symptoms; however, 62 (59.6%) of 104 patients with neovascularized plaques had neurological symptoms ($P<0.001$). In a

prospective study, the authors reported that SMI level was also a preferable marker in predicting the occurrence of future stroke with an AUC of 0.878 (22). These findings demonstrated that IPN detected by SMI plays a key role in plaque vulnerability and the occurrence of stroke, and

plaque neovascularization may be a potential intervention target for stroke prevention.

SWE is considered to be less operator dependent and to have higher reproducibility than earlier ultrasound elastography techniques based on operator compression of the tissue to induce transient stress and assess tissue deformation (stiffness). A previous study showed that the reproducibility of YM measurements quantified by the interframe coefficient of variation was 22% within the vessel wall and 19% within the carotid plaque (23). Our study presented a preferable agreement (ICC = 0.911, 95% CI, 0.848–0.948) for the assessment of PS using SWE between 2 independent sonographers.

SWE provides additional information related to plaque tissue stiffness, such as the presence of IPH and a large lipid core, which may indicate plaque vulnerability. Dahl *et al.* (27) reported that the stiffness of collagenous fiber was higher than that of lipids, and calcification had the highest stiffness among the plaque components. The mean YM values of representative plaques in the asymptomatic group were significantly higher than those in the symptomatic group (78.5 ± 25.4 vs. 51.5 ± 18.3 kPa). Ramnarine *et al.* (23) reported that the mean YM values of asymptomatic plaques and symptomatic plaques were 88 and 62 kPa, respectively, and these values were comparable to the findings obtained in our study. The regions of interest (ROIs) they selected were more than two circles each with 2-mm radii to sample the mean YM of the plaques randomly. Although the method can avoid the effects of the presence of different components on the mean YM, it also is associated with the problem of selection bias. In our study, we used the trace tool to evaluate the whole-plaque mean YM; thus, it might be able to avoid the issue of selection bias. Moreover, a significant relationship was evident between the YM of plaques and the degree of lumen narrowing. However, this relationship may be the result of a confounding factor as the severity of stenosis of symptomatic plaques was usually higher than that of asymptomatic plaques, and the mean YM of asymptomatic plaques was significantly higher than that of symptomatic plaques.

The assessment of the clinical potential of the advanced ultrasound techniques used in this study suggested that PS and IPN level were crucial markers for discriminating symptomatic plaques. SWE estimates of YM were a better marker than the IPN level, and combining the YM with IPN grades yielded improved discrimination performance (AUC = 0.89). Therefore, compared with the degree of stenosis, the additional information provided by SWE and

SMI imaging in atherosclerotic vascular diseases may help to improve individual risk stratification.

Further studies are needed to validate our primary findings, to extend the study to quantitative analysis of IPN and to assess YM heterogeneity within plaques, which may further help to precisely identify vulnerable carotid plaques. Advances in the SMI technique should be developed to overcome the limitations of conventional Doppler ultrasound, which enables the visualization of IPN without the use of intravenous contrast. However, SMI in contrast to CEUS only can be assessed with a specific ultrasound system of Canon Aplio at present. The development of SWE technology, such as improving the frame rate, should improve the reliability and enable evaluation of temporal variation in tissue stiffness throughout the cardiac cycle. Our study design used clinical ischemic symptoms as a surrogate measure of vulnerable plaques. Large-scale multicenter studies with long-term follow-up are needed to assess the predictive performance and prognostic ability of our findings in identifying vulnerable plaques and the risk of stroke. These findings would provide the evidence required for implementation of SMI and SWE imaging techniques as routine vascular ultrasound examinations.

Conclusions

Advanced ultrasound techniques, including SWE and SMI, are able to identify features of vulnerable carotid plaques with preferable reproducibility and high specificity and sensitivity. Advanced ultrasound characteristics of vulnerable plaques are well correlated with patient ischemic symptoms. Prospective randomized trials are needed to validate that criteria observed with advanced ultrasound techniques are predictive of ischemic events and that advanced ultrasound techniques allow better patient selection for revascularization therapies.

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Footnote

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Data Sharing Statement: Available at <http://dx.doi.org/10.21037/cdt-20-876>

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/cdt-20-876>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of the Beijing Tiantan Hospital (IRB No. KY2019-113-01). All participants gave written informed consent before ultrasound examinations.

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