ORIGINAL ARTICLE

Quantitative characterisation of carotid atherosclerotic plaque neovascularisation using contrast-enhanced ultrasound imaging: A feasibility study

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ABSTRACT

Introduction: Contrast-enhanced ultrasound (CEUS), an in vivo imaging tool for evaluating intraplaque neovascularisation (IPN), is an increasingly researched marker of susceptible atherosclerotic plaque. This study aims to assess the feasibility of quantifying carotid IPN using CEUS and to identify and characterise the neovascularisation in carotid plaques. The hospital's ethical committee approved the study, and the informed individual consent form of CEUS was obtained from all patients before the examination.

Materials and Methods: Seventy-one patients with carotid atherosclerotic plaques (95 plaques) were studied on CEUS. Contrast enhancement in the plaque was evaluated with visual interpretation and quantitative analysis. The intraplaque neovascularisation (IPN) test was graded on a 3-point scale. IPN was quantified using dedicated software for CEUS image analysis.

Results: It was found that the CEUS quantitative parameters were significantly different for plaques with varying types of echoes. The quantitative parameters also differed in soft, hard, and mixed plaques. The quantification of carotid IPN using CEUS was found feasible. The quantitative parameters measured from CEUS provide multiple references for carotid IPN of different echo types. This can help identify and monitor unstable atherosclerotic plaques.

Conclusion: CEUS has the potential to be an important tool in clinical application, specifically for diagnosing carotid atherosclerotic plaque features and vulnerability.

KEYWORDS:
Arteriosclerosis, Atherosclerotic plaques, Contrast-enhanced ultrasound, Contrast sensitivity, Neovascularisation

INTRODUCTION

Atherosclerosis is characterised by the constriction of arteries due to lipid and calcium-forming plaques that impede the normal flow of oxygenated blood and increase the risk of cardiovascular events such as myocardial infarction, stroke, and transient ischemic attack.[1] The latest report shows that about 270 million people in China currently have carotid atherosclerosis, and 200 million have carotid artery plaques.[2] Neovascularisation, the growth of microvascular networks within plaques, plays a critical role in plaque vulnerability and increases the risk of rupture, leading to adverse cerebrovascular events.[3] It has been established that plaque vulnerability and intraplaque neovascularisation (IPN) are related to the progression of atherosclerotic disease. Neovascularisation is the emergence of functional microvascular networks perfused by red blood cells. The body promotes the growth of blood vessels to supply the tissue that forms within an arterial wall, plaques, by providing a place for these tissues to grow. Recent advances in contrast-enhanced ultrasonography (CEUS) have demonstrated that ultrasound contrast agents allow for visualising these small microvasculature networks with a slow flow.[1]

Invasive and non-invasive testing techniques are the primary categories of carotid plaque examination techniques. Ultrasound, magnetic resonance imaging (MRI), computed tomography angiography (CTA), digital subtraction angiography (DSA), and other techniques are used to assess carotid plaque. DSA is the "gold standard," but it is invasive, expensive, and uses ionising radiation. It also cannot evaluate the wall and plaque interior.[4] CTA offers more intuitive images through three-dimensional reconstruction technology and is more sensitive to intra-plaque calcification. However, apart from its inability to detect neovascularisation in plaque, it also uses ionising radiation and there is a possibility of allergic reactions in patients due to the use of contrast material. Whereas MRI takes a long scanning time, as well as significantly impacted by motion and breathing artifacts. High-resolution magnetic resonance imaging (HRMRI) is time-consuming, expensive, not conducive to follow-up, and not suitable for some patients with non-MRI compatible metal implants. Carotid artery ultrasonography is currently the most commonly used clinical method to identify carotid plaques. CEUS uses microbubble contrast agents to reveal blood flow and neovascularisation in plaques. Fleiner et al.[5] found a more significant occurrence of new capillaries in the plaques of symptomatic individuals by comparing 22 patients

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displaying clinical symptoms with 27 patients having asymptomatic carotid atherosclerotic plaques. Contrast agents are restricted to the microvasculature and can enter the microvasculature network. Therefore, the appearance of microbubbles within the plaque is a manifestation of local neovascularisation. CEUS allows real-time, non-invasive observation of blood flow in plaques using sulphur hexafluoride microbubbles.

A study performed by Sedding et al.[6] highlighted the connection between neovascularisation in the plaque and the risk of haemorrhage and inflammation, both of which are major contributors to ischaemic stroke. Vulnerable plaque, known as unstable plaque, is strongly associated with ischemic stroke and transient ischemic attack (TIA).[7] Intraplaque neovascularisation (IPN), characterised by weak, immature vessels, is a marker of plaque instability and increases the likelihood of rupture and bleeding.[8] Routine ultrasound examination of the carotid artery can detect the thickness of arterial intima-media and the existence and size of the plaque. However, it cannot show the new blood vessels in the plaque well, and the density of new blood vessels in the plaque is closely related to the stability of the plaque. Contrast-enhanced ultrasound (CEUS) as intravascular tracers can display neovascularisation in plaques, making up for the shortcomings of conventional ultrasound examinations. CEUS is a new technique for evaluating neovascularisation in carotid plaques. Compared to traditional ultrasound diagnosis, CEUS technology significantly increases resolution sensitivity and specificity by enhancing dispersed echoes with contrast agents.[9-10] CEUS can display micro-vessels and blood perfusion in real time, reflect the blood perfusion of the neovascular through the degree of contrast enhancement in carotid atherosclerotic plaque, and identify the density of neovascular enhancement in plaques. This study mainly aims to analyse the neovascularisation in carotid plaques with different types of echoes quantitatively based on current technology, CEUS.

MATERIALS AND METHODS

Participant Recruitment

A total of 71 patients with carotid atherosclerotic plaques (95 plaques) who were diagnosed and treated in Yichang Second People's Hospital, Hubei, China, from May 2022 to May 2023, were selected as the study subjects. They comprised 42 males and 29 females; their ages ranged from 47 to 81 years, with a mean of 62.24±8.73 years. Subjects were selected based on the following inclusion and exclusion criteria.

The inclusion criteria are: 1) Carotid atherosclerotic plaque formation with a thickness ≥ 1.2mm was found by ultrasonography; 2) Those aged ≥30 years old. The decision to include patients 30 years old and above only was based on several key considerations. Firstly, there are low prevalence of carotid atherosclerotic plaque in young adults. Secondly, it is based on the institutional patient demographics in which our hospital, a specialized geriatric and chest pain centre, primarily serves an older patient population. As such, the majority of patients presenting to our institution for vascular evaluation are typically older than 30 years of age. Thirdly, we aimed to investigate the clinical implications of carotid plaque neovascularization in a group at higher risk for cardiovascular events, such as stroke and myocardial infarction. While it is acknowledged that early-onset atherosclerosis can occur, it is relatively rare and often associated with specific risk factors, such as familial hypercholestrolaemia or systemic inflammatory diseases. Given the low prevalence and distinct clinical presentation of early-onset disease, it was determined that the inclusion of younger patients would not significantly contribute to the primary objectives of this study.

Whereas the exclusion criteria are 1) Those who had cerebrovascular diseases in the past three months; 2) Those with complete occlusion of the common carotid artery or occlusion of the internal carotid artery; 3) Those with cardiac, pulmonary, and renal insufficiency. Cardiac insufficiency is defined as an ejection fraction (EF) of the left ventricle of less than 50%. Renal insufficiency is defined as elevated blood creatinine, elevated cystatin C, and decreased glomerular filtration rate on laboratory tests; Pulmonary insufficiency is defined as a ratio of first-second expiratory volume on exertion of less than 50 percent of the predicted value on pulmonary function testing; 4) Those who are allergic to ultrasound contrast agents; 5) Those with psychiatric disorders who are unable to complete the study; 6) Those who are pregnant or breastfeeding; 7) Those who have carotid plaque calcifications involving greater than 30% of plaque area. This study obtained approval from the Research Ethics Committee of Yichang Second People's Hospital, Hubei, China. All subjects were informed of the inspection process and signed the informed individual consent forms for the contrast-enhanced ultrasound before the examination.

Conventional US Imaging

An Aplio 500 ultrasound system, as shown in Figure 1 (Toshiba, Canon Medical Systems Corporation, Tokyo, Japan), was used with a high-frequency probe (frequency at 4 - 11MHz), and the mechanical index was set at 0.08, the frame frequency was 12 frames/second, the depth was around 3 - 5cm. During the examination, the individual was examined by lying in a supine position with the head turned 45 degrees to the contralateral side. The American Society of Echocardiography's consensus scanning procedure was used to acquire images.[11] The patient was positioned supine to evaluate both left and right carotid arteries, scanning from the common carotid artery through the carotid bifurcations with a focus on the internal and external carotid arteries on both sides using two-dimensional ultrasound.

Each anatomical region was assessed from a variety of angles. The gain and imaging depth were modified individually for each patient to achieve optimal ultrasound images. Plaques were thoroughly checked for presence on both sides. Three images with clear echoes of plaques from both the cross-sectional and longitudinal sections were selected to record the plaque's size, shape, position, echo, and edge, and the images were saved to the system. After the two-dimensional images of the plaque were displayed, the probe was fixed on the plaque for local amplification, and the carotid intima-media thickness (IMT) and the length of the carotid artery plaque were measured. The target plaque was counted three times, and the average of three measurements

was taken and recorded in the system as a primary measurement before CEUS.

CEUS Imaging

The CEUS imaging was performed using the same ultrasound system mentioned in the previous section. The carotid plaque CEUS examinations were performed by two diagnostic ultrasonographers who had each been trained in both ultrasound diagnosis work for more than ten years and CEUS for more than five years. They jointly confirmed the outcomes and reached a consensus on judgments. The diagnostic ultrasonographers were blinded to the history of all subjects, and they kept the subjects' information strictly confidential. Two Aplio 500 ultrasound systems of the same configuration were used for this study.

A freeze-dried powder contrast agent, SonoVue (Bracco Suisse SA, Milan, Italy), was employed, as shown in Figure 2A. Each bottle contains 59 mg of SF6 (sulfur hexafluoride) gas and 25 mg of freeze-dried powder. The contrast agent powder and 5 ml of normal saline were mixed into a suspension liquid, as shown in Figure 2B. Then, 2.4 ml of the suspension was aspirated and pushed into the patient's body through the cubital vein in 2 - 3 seconds using an 18-gauge intravenous cannula. Then, 5 ml of normal saline was injected to flush the injection channel. The contrast effect was observed within the carotid artery lumen 15 to 30 seconds following injection, allowing for the capture and preservation of high-quality contrast images for approximately 1 minute post-administration.

The area of interest was continuously imaged for approximately 90 seconds using the dual-screen contrast mode of two-dimensional ultrasound and CEUS. The images were saved for further analysis, as illustrated in Figure 3, and stored digitally in DICOM (Digital Imaging and Communications in Medicine) format for later offline examination. The quantitative analysis software, specifically the Time Curve Analysis (TCA) function equipped with the ultrasound instrument, was used to generate a time-intensity curve (TIC); if multiple regions of interest were observed in the same patient, the interval between the second CEUS should be at least 15 minutes. Subjects were monitored for 30 minutes before they were allowed to leave in case any complications developed.

Observation Indicators

According to the contrast distribution in the plaque, the semiquantitative visual grading standard for intraplaque neovascularisation is based on the following rules[12]: Grade I: no enhancement, no microbubble contrast agent in the plaque; Grade II: a minor enhancement, a small amount of microbubble contrast agent in the plaque, see punctate, little flake-like enhancement; Grade III: massive enhancement, a large amount of microbubble contrast agent can be seen in the plaque.

Plaques were characterised by their appearance on standard ultrasound images. They were classified according to widely used criteria as follows[13]: (a) Soft plaques with echogenicity lower than that of the surrounding adventitia for over 80% of the plaque area, without acoustic shadowing; (b) Hard plaques with echogenicity equal to or greater than that of the surrounding adventitia for over 80% of the plaque area, without acoustic shadowing; (c) Calcified plaques containing over 90% circumferential calcification and displaying as bright echoes within the plaque along with acoustic shadowing; (d) Mixed plaques containing less than 90% circumferential calcification or having associated echo-dense and anechoic regions occupying less than 80% of the plaque area.[13] In this study, we enrolled subjects with soft, hard, and mixed plaques. Because calcified plagues with calcifications involving greater than 30% of plaque area are excluded as they are challenging to analyse by the time-intensity curve (TIC) quantitative assessment.

Then, for quantitative assessment, firstly, a stable continuous dynamic image was chosen. Next, the region of interest (ROI) was selected, with one ROI being placed in the plaque as the evaluation ROI and the other in the lumen as the corresponding match between the two. The plaque's border was traced following its shape to prevent other areas from being covered. A rectangular sampling frame placed in the middle of the carotid lumen where the plaque was located would be the reference area (intraluminal). Then, the time-intensity curve (TIC) was computed and produced by the software.

The main quantitative analysis parameters of TIC were the peak intensity (PI), baseline intensity (BI), enhancement intensity in the arterial lumen, and time to peak is the time from the start of intravenous injection of contrast agent to the time when the maximum intensity is reached in the region of interest (TTP), mean transit time (MTT), plaque enhancement intensity (EI) = PI - BI, Ratio value = EI/enhancement intensity in the arterial lumen. The EI parameter measures the SonoVue intravascular tracer's intensity variations between pre- and post-injections within the plaque ROI.

Statistical Analysis

In this study, data analysis was performed using SPSS 22.0 statistical software (IBM, USA). The normality of measurement data was tested using the Shapiro-Wilk method, and normally distributed data were expressed as mean ± standard deviation. Group comparisons were conducted using analysis of variance (ANOVA), and pairwise comparisons between groups were performed using the LSD method. Categorical data were presented as counts (percentages) and analysed using the chi-square test or Fisher's exact test. A two-tailed test was used, and differences were considered statistically significant at $p<0.05$.

RESULTS

A single carotid ultrasound and CEUS examination typically take around 30 minutes. The images of carotid plaque acquired through conventional ultrasound are depicted in Figure 3, while those obtained using CEUS are presented in Figure 4.

Among 71 patients with carotid atherosclerosis, 95 plaques were identified as more than 1.2 mm thick, including 46 soft plaques, 37 mixed plaques, and 12 hard plaques. The enhancement rates (Enhancement rate = (grade II + grade III)/total number of cases × 100%) of plaques with different echo types were as follows: soft plaque contrast-enhancement

Table I: Semiquantitative visual grading standard for different plaque types and the enhancement rates

Groups	Numbers	Grade I	Grade II	Grade III	Enhancement rate	χ^2	p-value
Soft plaques	46	2	16	28	95.7%*	13.291	<0.001
Mixed plaques	37	5	18	14	86.5%*		
Hard plaques	12	6	5	1	50.0%		

Note: Compared with the Hard plaques group, *p<0.05

Table II: Comparison of EI values for different plaque types

Plaque types	Soft plaque (n=46)	Hard plaque (n=12)	Mixed plaque (n=37)	F-value	p-value
EI value (dB)	11.95±3.33*	5.47±1.36*	7.76±2.21*	38.540	<0.001
P (compared with soft plaque)		<0.001	<0.001		
P (compared with hard plaque)			0.013		

*Values are stated as mean ± standard deviation

Table III: Comparison of Ratio values for different plaque types

Plaque types	Soft plaque (n=46)	Hard plaque (n=12)	Mixed plaque (n=37)	F-value	p-value
Ratio value	0.53±0.18*	0.27±0.09*	0.32±0.11*	26.241	<0.001
P (compared with soft plaque)		<0.001	<0.001		
P (compared with hard plaque)			0.320		

*Values are stated as mean ± standard deviation

Table IV: Comparison of TTP values for different plaque types

Plaque types	Soft plaque (n=46)	Hard plaque (n=12)	Mixed plaque (n=37)	F-value	p-value
TTP（s）	18.28±1.98*	26.79±2.11*	9.44±1.69*	453.039	<0.001
P (compared with soft plaque)		<0.001	<0.001		
P (compared with hard plaque)			<0.001		

*Values are stated as mean ± standard deviation

Table V: Comparison of MTT values for different plaque types

Plaque types	Soft plaque (n=46)	Hard plaque (n=12)	Mixed plaque (n=37)	F-value	p-value
MTT（s）	7.65±1.43*	28.03±2.03*	22.81±3.02*	659.229	<0.001
P (compared with soft plaque)		<0.001	<0.001		
P (compared with hard plaque)			<0.001		

*Values are stated as mean ± standard deviation

accounted for 95.7%, mainly manifested as grade II enhancement and grade III enhancement, followed by mixed plaques (86.5%); hard plaques accounted for the least amount (50.0%), mainly showing grade I enhancement and grade II enhancement, as shown in Table I. Fisher's exact probability method was used, and the difference in enhancement rates between the three groups was statistically significant, with the Hard plaques group having a significantly lower enhancement rate than the other two groups (p<0.05).

Based on CEUS images, the quantitative parameter values of CEUS images were found to be different in soft, hard, and mixed plaques. The EI and Ratio values of soft plaques were significantly higher than those of hard plaques and mixed plaques (p<0.05); the TTP and MTT of hard plaques were significantly higher than those of soft plaques and mixed plaques (p<0.05), the TTP of soft plaques was significantly higher than that of mixed plaques (p<0.05), the MTT of soft plaques was significantly lower than that of mixed plaques (p<0.05) as shown in Table II – V.

DISCUSSION

SonoVue has been demonstrated to be safe, as it does not become trapped in small blood vessel networks and does not spread across vascular or micro-vessel walls. There is no evidence of any adverse effects associated with SonoVue.[14] In a 49,100 case study analysing the safety of adverse events with SonoVue, it was noted that SonoVue had a favourable safety profile, with a low incidence of adverse events, most of which were mild and of short onset and duration.[15] In another study conducted on 502 children, Sonovue contrast was found to be safe, feasible, diagnostically reliable, and

Fig. 1: Canon Aplio 500 ultrasound system

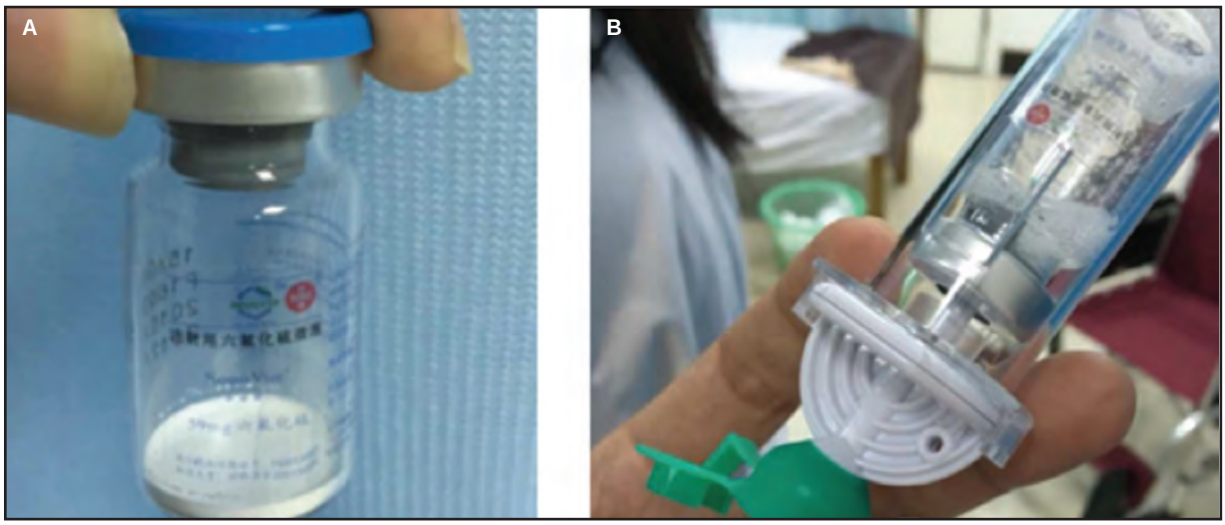

Fig. 2: Contrast agent SonoVue.
(A) Freeze-dried powder is a form of contrast agent stored in a bottle. (B) The contrast agent powder is mixed with normal saline.

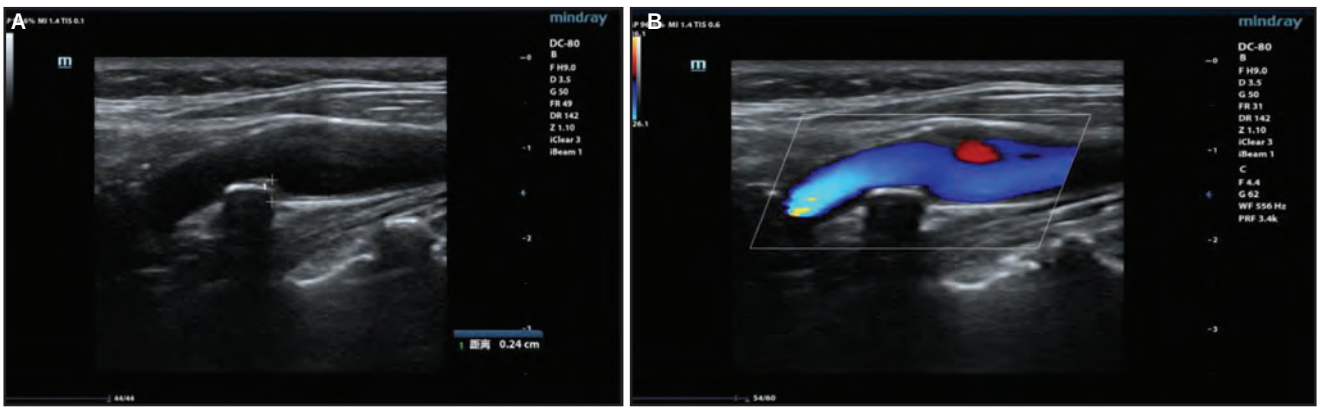

Fig. 3: Conventional ultrasound of carotid mixed plaque.
(A) Two-dimensional ultrasound image of a mixed echogenic plaque in the longitudinal section of the internal carotid artery. (B) Colour Doppler image of the same mixed echogenic plaque in the longitudinal section of the internal carotid artery.

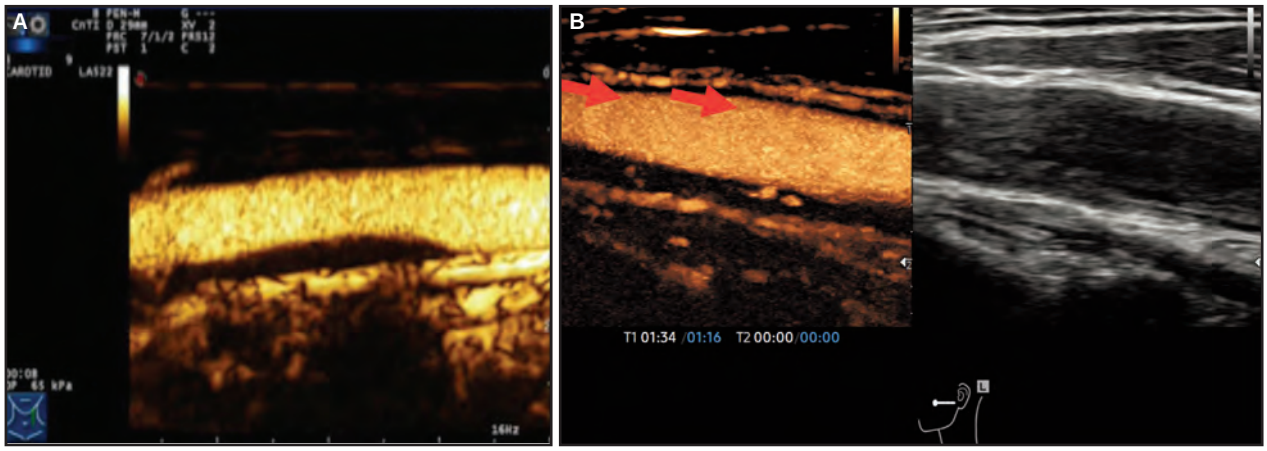

Fig. 4: Contrast-enhanced ultrasonography of carotid soft plaque.
(A) Longitudinal image of the common carotid artery shows an unobvious contrast agent in the soft plaque using the contrast mode. (B) A large amount of contrast agent (Red arrow) is seen inside the plaque of the longitudinal image of the common carotid artery using the dual-screen contrast mode of two-dimensional ultrasound and CEUS.

effective.[16] Sulphur hexafluoride gas is mainly excreted from the body via respiration through the pulmonary circulation within 15 minutes of injection. In CEUS imaging, microbubbles exhibit a nonlinear response to ultrasound insonification due to their high compressibility and resonance, distinguishing them from tissue's linear response. This distinct behaviour allows for differentiation between tissue and microbubble responses.

Soft plaques are also called unstable plaques, prone to rupture, thrombosis, and myocardial infarction. Hard plaques are also called stable plaques, which are more durable than soft plaques and less prone to local rupture and thrombus formation. Mixed plaques are plaques that have the characteristics of soft plaques and hard plaques, presenting mixed echoes. Among the 95 plaques identified in our study, it was found that there were 46 soft plaques, 12 hard plaques, and 37 mixed plaques, indicating that there were relatively more unstable plaques in patients with carotid atherosclerosis. The distribution of CEUS enhancement grading of carotid plaques of different echo types showed that the lower the plaque echo, the more obvious the CEUS enhancement was, and the proportion of contrast enhancement in soft plaques was the highest, accounting for 95.7%, with grade II and III enhancement as the major part; mixed plaques were the second, with a contrast enhancement of 86.5%, and hard plaques accounted for the lowest proportion of enhancement, accounting for 50.0%, with grade I and II as the major part. The difference in the distribution of CEUS enhancement grades of carotid plaques of different echo types was statistically significant ($p<0.05$).

This also shows that soft and mixed plaques are more likely to be enhanced by CEUS, while hard plaques are less likely to be enhanced. This is because the interior of soft plaques is mainly composed of lipids and cholesterol, while the interior of hard plaques is mainly composed of calcification. Lipid necrosis can enhance inflammatory activity within atherosclerotic plaques, thereby inducing the formation of new blood vessels. Soft and mixed plaques have more blood vessels than hard plaques, which greatly increases the vulnerability of plaques. Therefore, such plaques need further examination to clarify their stability. Accurately assessing the nature of plaques can better guide the dosage of drugs, this is of great clinical value.

The results of preclinical studies have shown that there is a direct relationship between plaque enhancement and neovascularisation,[17] which has also been confirmed in clinical practice. Giannoni et al. (2009) found that plaque intraplaque enhancement was associated with an increase in small immature micro-vessels (20 – 30 mm diameter).[18] The ultrasonic contrast agent caused a rise in the intra-plaque signal intensity. In our study, the enhanced intensity of CEUS for soft, mixed and hard plaques was (11.95±3.33) dB, (7.76±2.21) dB, and (5.47±1.36) dB, respectively. The enhanced intensity ratios were 0.53±0.18, 0.32±0.11, and 0.27±0.09, respectively. The EI and Ratio values of soft plaques were significantly higher than those of hard plaques and mixed plaques ($p<0.05$). The distribution of CEUS enhancement quantitative parameters of carotid plaques of different echo types also showed that the lower the plaque echo, the more obvious the CEUS enhancement was. This result showed a correlation between visual grading of intraplaque enhancement and quantitative analysis of computed enhancement intensity based on CEUS. Plaques with high CEUS visual grading showed increased EI and ratio values, as displayed in the Tables. These findings are consistent with previous research results.[17]

Additionally, our findings indicate that soft plaques exhibit more pronounced contrast enhancement than other plaques. This aligns with the results of prior studies.19 Among the cohort of patients with soft plaques, a more significant proportion exhibited contrast enhancement upon visual interpretation and more pronounced enhancement as evaluated through EI and ratio value analysis. This discovery implies that CEUS has the potential to detect highly vascularised and vulnerable soft carotid plaques.

CEUS shows that the formation of neovascularisation in carotid plaques has a good correlation with the results of histological examination, and it is best to use quantitative software to analyse and evaluate. However, its role in daily clinical practice still needs further confirmation.[4] The TIC can be used to quantitatively analyse the neovascularisation inside the carotid plaque, effectively avoiding the measurement bias caused by subjective factors in the semiquantitative visual grading.[20] CEUS quantitative parameters can reflect the pathological characteristics of atherosclerotic plaques. PI can reflect the density of new blood vessels in the plaque, and the higher the PI, the greater the density of new blood vessels.[21] TTP mainly reflects the perfusion pattern of neovascularisation in the plaque, in which the more minor the TTP, the faster the perfusion rate.[22]

Clinical studies have shown that increased intra-plaque neovascularisation is an independent risk factor for the progression of atherosclerosis and an essential feature of vulnerable plaques.[8] Therefore, the larger the PI, the smaller the TTP, and the higher the plaque vulnerability. In our study, the EI value and Ratio value of soft plaque were significantly higher than those of hard plaque and mixed plaque ($p<0.05$), the TTP of hard plaques were significantly higher than those of soft plaques and mixed plaques ($p<0.05$), these results are highly consistent with previous studies above.

Our results also confirm that soft plaques are the most vulnerable plaques, which means the TIC curve can efficiently and accurately identify and characterise unstable carotid plaques. Based on CEUS imaging quantitative parameters, a more reliable reference value for clinical diagnosis of the nature of carotid atherosclerotic plaques and plaque vulnerability is offered. Our study has certain limitations, primarily due to its single-centre design. As the hospital where the study was conducted is a geriatric facility with specialised stroke and chest pain centres, most patients are those with underlying conditions such as diabetes, coronary heart disease or hypertension. This may introduce a degree of selection bias in our findings. Therefore, our results may differ from those of large-scale multicentre studies from other academic institutes. Our research is still clinically significant, and further in-depth investigations will be conducted.

CONCLUSIONS

Based on the safety of Sonovue as well as the reasonable scan time of 30 minutes, it was found that performing CEUS imaging of carotid IPN at our hospital is feasible when carotid plaque stability needs to be assessed for clinical benefits. CEUS imaging allowed better identification and quantification of carotid IPN through quantitative parameters, specifically EI, Ratio Values, TTP, and MTT. Quantitative CEUS parameters can provide additional information on plaque vulnerability based on semi-quantitative visual assessment, which can help in patient screening and appropriate interventions. CEUS can quantitatively evaluate the intensity of neovascularisation inside plaques of different echo types and thus provide a reference for clinical diagnosis of the nature of carotid atherosclerotic plaques and plaque vulnerability.

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DISCLOSURE STATEMENT

The authors report there are no competing interests to declare.

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