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Evaluation of carotid plaque vulnerability with different echoes by shear wave elastography and CEUS

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Abstract

Objective: To examine carotid plaques with different echoes by Shear Wave Elastography (SWE) and Contrast Enhanced Ultrasound (CEUS), and to investigate reliable methods to quantify the characteristics associated with carotid vulnerable plaques, providing an important basis for quantitative assessment of plaques.

Methods: 2D ultrasound, SWE and CEUS were performed on 244 carotid plaques, and the echoes were evaluated according to the Gray-Weale classification scale and Gray-Scale Median (GSM), and the mean Young's Modulus (YM) of the plaque was measured and the intra-plaque neovascularization was observed to investigate the relationship between carotid plaque types with different echo characteristics, GSM and the values of each parameter of YM and CEUS. The relationship between GSM and YM and CEUS values was investigated.

Results: The differences between GSM values ($F = 49.742$, $P < 0.001$), with the maximum, mean, and minimum YM values of ultrasound elastography ($P < 0.001$), and with the number ($P < 0.001$) and density ($P = 0.047$) of neovascularization on CEUS were statistically significant for the different echogenic types of plaques, and the lower the echogenicity of the plaque, the lower the GSM values (r = 0.632, P < 0.001), the smaller the YM values (all $r > 0$, $P < 0.001$), and the higher the neovascularization number and density values ($r < 0$, $P < 0.001$); and there were also statistically significant differences between the above indicators in the vulnerable and stable plaque groups (all P < 0.05).

Conclusion: GSM, SWE, and CEUS techniques can quantitatively evaluate the vulnerability of different echo carotid plaques in a more comprehensive and objective manner, which may help clinical identification of vulnerable plaques, and provide important reference values for early diagnosis and treatment in clinical practice.

Keywords: Ultrasound; Carotid plaque; CEUS; GSM; Echo.

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Background

Stroke is a major worldwide public health problem with increasing morbidity, disability, and mortality rates year by year, and approximately 10-25% of strokes are caused by carotid atherosclerosis [1,2], and the treatment and management of patients with carotid atherosclerosis is largely influenced by the presence of clinical symptoms and the imaging assessment of the degree of stenosis of carotid plaques and plaque surface characteristics [3], without taking into account the presence of clinically unrecognizable stroke due to the presence of vulnerable plaque [4], and luminal embolism (due to plaque rupture or release of plaque contents into the bloodstream causing thrombosis) caused by plaque instability is more important in the pathogenesis of ischemic stroke than is cerebral insufficiency due to carotid stenosis or occlusion [5], which is closely related to intraplaque composition and is currently Clinically, plaque echogenic information is mainly assessed roughly by 2D ultrasound, which cannot quantify the fragility of the plaque. Some components within the plaque such as fibers, lipid core, calcification and hemorrhage show different GSM due to different acoustic impedance. Therefore, theoretically GSM can reflect the components contained within the plaque. Several studies [6,7] found higher GSM in plaques with predominantly fibrous and calcified, lower GSM in plaques with hemorrhage and lipid core, and a relationship between plaques with low median grayscale and histopathological features with a higher risk of cerebrovascular events was also confirmed [8], thus GSM is a computer-assisted measurement of plaque echo that can overcome the visual subjective evaluation of bias associated with plaques, but suffers from poor reproducibility [9] making it difficult to implement in the clinic, the reasons for this variability may be the variability in GSM values [10], inconsistent results obtained from GSM measurements using different software, lack of consensus in standardization methods, etc.; on the other hand, it is an average measurement of the overall plaque echogenicity and does not allow for a separate evaluation of possible high-risk areas within the plaque (prone to rupture) cannot be evaluated individually [11]. Therefore, this study applied CEUS and SWE to accurately and finely evaluate plaques with different echogenic types and to explore the relationship with the echogenic features, which in turn helps to determine the vulnerability of different echogenic plaques, contributes to further risk stratification, and provides some clinical references on preventive treatment.

Intraplaque Neovascularization (IPN) is an independent predictor of plaque vulnerability [12], while CEUS can more sensitively show the distribution and number of intraplaque neovascularization, and a significant correlation between IPN assessed by CEUS and microvessels on carotid plaque histology after denudation has been demonstrated [13], in addition, SWE as an emerging ultrasound imaging method, which uses acoustic radiation force to generate shear wave propagation in tissue and assesses tissue stiffness by quantifying Young's Modulus (YM), the purpose of this study was to quantify the echogenic characteristics of plaques, assess whether CEUS, SWE has the ability to identify vulnerable plaques, and collate multiple techniques to evaluate the vulnerability of plaques, which is crucial to optimize patient treatment and management, which not only helps to prevent This is essential to optimize the treatment and management of patients, not only to prevent stroke, but also to delay the cognitive and motor impairment caused by carotid plaque vulnerability.

Methods

Research subjects

March 2021 to May 2022 in the first hospital of Qinhuang dao of the diagnosis of acute ischemic stroke associated with ipsilateral carotid artery plaque, All patients underwent CEUS, SWE and other examinations, and gave informed consent and signed a consent form. This study had been approved by the Hospital ethics Committee.

Materials and methods

1. Imaging was performed using the Canon ultrasound system, ultrasound Imagine Aixplorer (Provence, France), using the SL10-2 probe, using image optimization Settings to standardize the procedure, and performed by the same doctor.

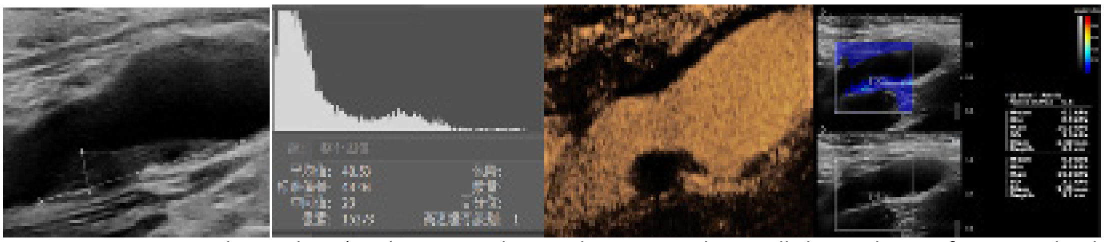
2. Examination method and Observation indicators

1) Echo classification: Conventional two-dimensional grayscale ultrasound visually classified carotid plaques into four groups: homogeneous hypoechoic (type I), have some calcification (<50%) of the plaque (type II), have some calcification (>50%) of the plaque (type III), and homogeneous hyperechoic (type IV) according to the plaque classification method proposed by Gray-Weale et al [14] with reference to the echogenicity of the sternocleidomastoid muscle. In this study, homogeneous hyperechoic plaques were not included because of their stability.

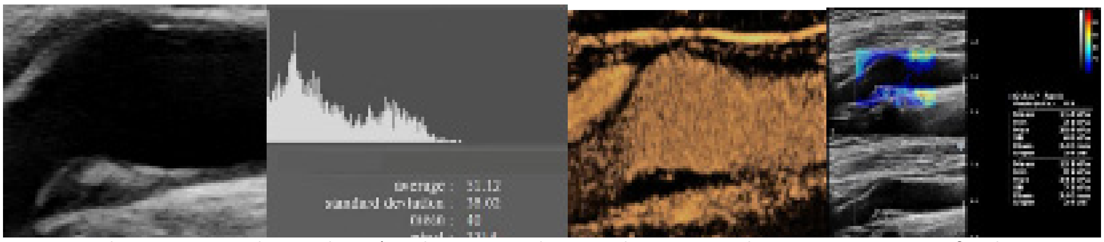
2) Median Gray Scale (GSM): The median gray value of the patch was further processed on the two-dimensional image and processing analysis was performed using Adobe Photoshop 7.0 (Adobe Systems Inc, San Jose, CA, USA) and two reference points were selected i.e. blood as 0 and extravascular membrane 195 [6]. For this purpose, the region of blood was selected as the reference structure for hypo echogenicity and assigned a gray scale value of zero and a portion of the extravascular membrane was selected as the reference structure for hyper echogenicity and assigned a value of 195. Then, carotid plaques were outlined in the normalized images and GSM was measured using the histogram function in the software to find the GSM values of the different echogenic classified plaques and compare them.

3) Shear wave elastography (SWE): SWE imaging mode was activated, the sampling frame included the measured plaque, the patient was instructed to hold his breath, and the probe was pressed as lightly as possible on the examined area during the test to ensure a clear image without exerting pressure on the neck tissue, and after the image was stabilized, the image was frozen and stored, and at least two regions of interest with a diameter of 2 mm were analyzed and each Q The maximum Young's modulus, minimum Young's modulus and average Young's modulus of each Q-Box were recorded. The average of the maximum Young's modulus of multiple Q-Boxes was taken as the maximum Young's modulus of the plaque, and the intermediate Young's modulus values and the minimum Young's modulus values were performed as above, all by the same sonographer.

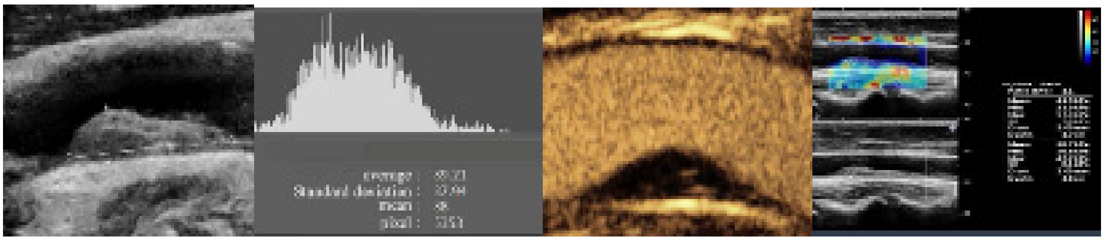
4) Contrast enhanced ultrasound (CEUS): In contrast mode, parameters and gain were adjusted and 1.6 ml of contrast agent (SonoVue; Bracco, Italy) was injected through the median cubital vein, followed by 5 ml of saline flush, and once the contrast agent was injected, recording of dynamic images was started for 2 min and saved, [15] the way the contrast agent diffuses from the outer membrane of the vessel to the base of the atheromatous plaque indicates intra-plaque neovascularization, and according to the study: The neovascularization was visually classified into 5 grades according to the grading criteria in the study [16]: Grade 0: no significant enhancement within the plaque; grade 1: Enhancement in the shoulder or the base; grade 2: enhancement visible in both the shoulder and the base; grade 3: Enhancement area moving towards the center of the plaque; grade 4: Diffuse enhancement or formation of ulcers within the plaque. The number of neovascularization was calculated as the most number of moving enhancement points on a cross section in CEUS, the density = number/plaque area, carotid plaque area was measured in two-dimensional ultrasound mode.



Type I: Homogeneous hypoechoic (median grayscale: 23; ultrasonography: small clumped area of anterior shoulder enhancement visible (grade 4); elastography: maximum, intermediate and minimum Young's modulus values of SWE averaged 12.7 kPa, 8.0 kPa, 2.8 kPa).



Type II: Inhomogeneous hypoechoic (median grayscale: 40; ultrasonography: punctate areas of enhancement (grade 2) are visible in both the shoulder and the base; elastography: maximum, intermediate and minimum Young's modulus values of SWE average 24.8kPa, 11.8kPa, 1.1kPa).



Type III: Inhomogeneous hyperechoic (median grayscale: 88; ultrasonography: punctate enhancement area visible only in the shoulder (grade 1); elastography: maximum, intermediate and minimum Young's modulus values of SWE averaged 52.3 kPa, 35.8 kPa, 29.9 kPa).

Statistical methods

SPSS 23.0 statistical software was applied, and the measurement data conforming to normal distribution were expressed as x ± s, and the non-normal data were expressed in the form of median (interquartile spacing); one-way ANOVA was used for the comparison of GSM and YM of different echogenic plaques, and the SNK-q method was used for the two-way comparison between groups; the comparison of ultrasonographic enhancement grading of different echogenic plaques was performed by Kruskal- Wallis H test and Nemenyi method for two-way comparison between groups; Pearson correlation coefficient was used to analyze the relationship between plaque GSM and YM and contrast parameters, and the difference was considered statistically significant at $P < 0.05$.

Results

Analysis of GSM values and distribution of different types of plaques

The GSM values of the three types of plaques increased according to the different echogenic types and were statistically significant (P<0.05) by F-test, with 99 homogeneous hypoechoic plaques (type I), 75 inhomogeneous hypoechoic plaques (type II) and 70 inhomogeneous hypoechoic plaques (type III), as shown in Table 1. Two-by-two comparison between groups: compared with type I plaques, the GSM values of type II and III plaques increased, and the difference was statistically significant (P=0.000); compared with the group of inhomogeneous hypoechoic plaques, the GSM values of the group of inhomogeneous hypoechoic plaques increased significantly, and the difference was statistically significant (P=0.000). The characteristics of the median gray distribution of the three different echogenic types of plaques are shown in Figure 2, and in further correlation analysis showed that there was a significant correlation between echotype and grayscale median values (*r*=0.527, *P*=0.000) (Figure 3).

Table 1: Comparison between different plaque echotypes and GSM values.

	Number of plaques	GSM
I	99 (40.6%)	31.1 ± 10.1
II	75 (30.7%)	41.3 ± 11.7a
III	70 (28.7%)	59.8 ± 20.3ab
F	49.742	
P	<0.001	

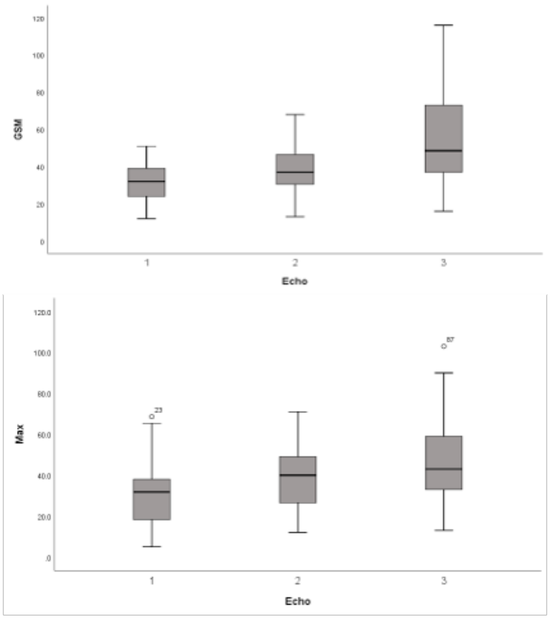


Figure 2: Distribution characteristics of median gray and stiffness maxima for different echo types (the dark line in the middle of the box is the median, the T-shaped bar represents the minimum and maximum values).

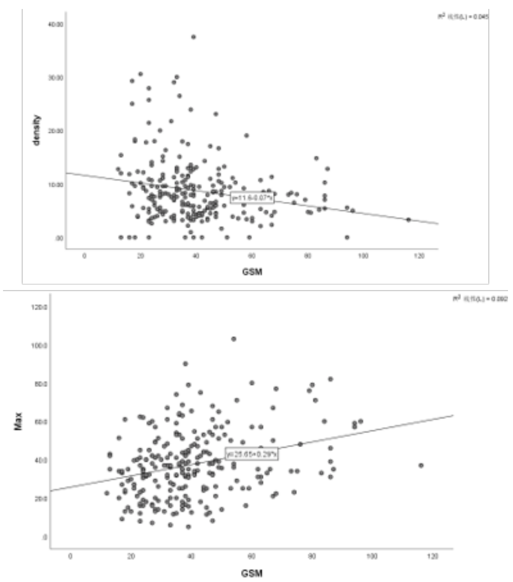


Figure 3: Distribution characteristics of median gray and stiffness maxima for different echo types (the dark line in the middle of the box is the median, the T-shaped bar represents the minimum and maximum values).

Comparison of elasticity values of different echogenic plaques

Statistical significance (*P* < 0.001) existed between different echotypes and plaque stiffness, and the mean Young's modulus values of the three were compared: type I < type II < type III (Table 2**)**, and the distribution characteristics are shown in Figure 2, and in the correlation analysis, a correlation was found between plaque echotype, GSM and YM (*P* = 0.000), i.e. the lower the GSM value, the more the echotype tends. The lower the GSM value (Figure 3), the more the echotype tends to be of lower level, and the smaller the stiffness value.

Table 2: Comparison of YM of carotid plaques with different echoes (x ± s).

	YM (kPa)			P
	Max	Mean	Min	
I	30.2 ± 14.1	18.9 ± 12.3	11.2 ± 11.8	<0.001
II	38.9 ± 14.4	29.9 ± 12.8	20.6 ± 11.7	<0.001
III	46.5 ± 20.0	39.3 ± 16.1	25.8 ± 11.8	<0.001

Comparison of ultrasonographic parameters

There was no statistically significant difference between the grading of ultrasonographic enhancement intensity (*P*=0.088) and plaque area (*P*=0.211) of different echogenic types of carotid plaques, and the proportion of type II was the highest in all three echogenic types (49.5% for type I, 56% for type II, and 52.9% for type II), and the enhancement grades in the homogeneous hypoechoic and inhomogeneous hypoechoic plaque groups were mostly grade 2 and 3, but there were also homogeneous hypoechoic plaques that showed no enhancement; the enhancement grade in the inhomogeneous hypoechoic plaque group was mostly grade 1 and 2. The plaques in the three groups with different echotypes did not show significant differences with neovascular grading; however, there were statistically significant differences between the echotypes and the number (*P*=0.000) and density (*P*=0.047) of neovascularization (Table 3), and two comparisons between the groups: compared with the homogeneous hypoechoic Compared with the homogeneous hypoechoic plaque group, the number and density of neovascularization were significantly lower in the inhomogeneous hypoechoic plaque group and the inhomogeneous hypoechoic plaque group, with statistically significant differences (*P* < 0.05); the number and density of neovascularization in the inhomogeneous hypoechoic plaque group were also lower than those in the inhomogeneous hypoechoic plaque group, with statistically significant differences (*P* < 0.05). Correlation analysis of echo characteristics with ultrasonographic parameters showed that there was a negative correlation between echo type and neovascularization number and density, while there was a positive correlation between GSM value and neovascularization density only. The above images illustrate different GSM, CEUS and elastography patterns for the three types of patch echo (Figure 1).

Table 3: Comparison of different echogenic plaques in ultrasonography mode.

		Stable	Unstable	T/Z	P
	I	65 (26.6%)	34(13.9%)		
	II	57 (23.4%)	18(7.4%)		
	III	57 (23.4%)	13(5.3%)	2.384	0.018
	GSM	41.00 (23)	35.00 (19)	-7.165	<0.001
Type	number	5.00 (3)	8.00 (3)	-8.047	<0.001
	denisty	6.90 (5.18)	10.14 (6.04)	-4.158	<0.001
CEUS	Max	39.00 (20)	25.00 (10.5)	-5.68	0.001
	Mean	27.40 (22.1)	21.00(15.8)	-3.559	<0.001
SWE	Min	18.10 (17.0)	10.80 (15.5)	-3.559	<0.001

Ultrasound multiparametric comparison of stable and vulnerable plaques

Previous studies have shown that CEUS scores of 0, 1 and 2 are mostly stable plaques, while those of 3 and 4 are mostly vulnerable plaques. Based on this theory for further research [17], and a comparative study was performed in terms of echo characteristics, ultrasonography and elastography, and there were significant differences between the vulnerability of plaques and the type of echo and median grey scale (P=0.018), and in terms of plaque distribution, the vulnerable plaque group, compared to the stable group, showed a homogeneous low more echogenicity and inhomogeneous hypoechogenicity and less inhomogeneous hypoechogenicity, presenting lower GSM values; on CEUS, the number and density of neovascularization were higher in the vulnerable group, but there was no statistical difference between them and the grading; and they presented lower Young's modulus values in elasticity (Table 4).

The AUC under the curve for the combined prediction of echotype, grayscale median, stiffness maximum and neovascularization and density in determining vulnerable plaques were 0.594, 0.802, 0.739 and 0.855, respectively, and the combined prediction of ultrasonography was higher than that of single ultrasound elastography, echotype and grayscale median, and the AUC of echotype was the lowest, and the sensitivity and specificity were also significantly lower than those of other methods, with statistically significant differences (P<0.001), as shown in Figure 4 and Table 5; meanwhile, this study found that the thresholds for median grayscale, elastic maximum, number of neovascularization and density were 35.5, 29.4, 6.5 and 8.7, respectively.

Table 4: Comparison of echotypes and GSM values in the stable plaque group and the vulnerable plaque group.

	CEUS							
	Type (Grade)					Number	Area	Density
	0	1	2	3	4			
I	7	6	49	29	8	6.0 (4)	0.73 (0.45)	8.45 (8.27)
II	0	14	42	19	0	6.0 (3)	0.73 (0.58)	7.94 (5.51)
III	4	17	37	9	3	4.5 (3)	0.67 (0.48)	6.45 (5.59)
P			0			0.003	0.211	0.047

Table 5: Diagnostic efficacy of echo, elastography, and ultrasonography in determining vulnerable plaques (%).

	AUC	Sensitivity	Sensitivity	OR	P
Echo Type	0.594	63.90%	53.10%	(0.514, 0.675)	<0.001
GSM	0.802	70.60%	82.80%	(0.741, 0.862)	<0.001
Stiffness Max.	0.739	79.40%	64.10%	(0.671, .808)	<0.001
neovascularization	0.855	81.10%	79.70%	(0.796, 0.915)	<0.001

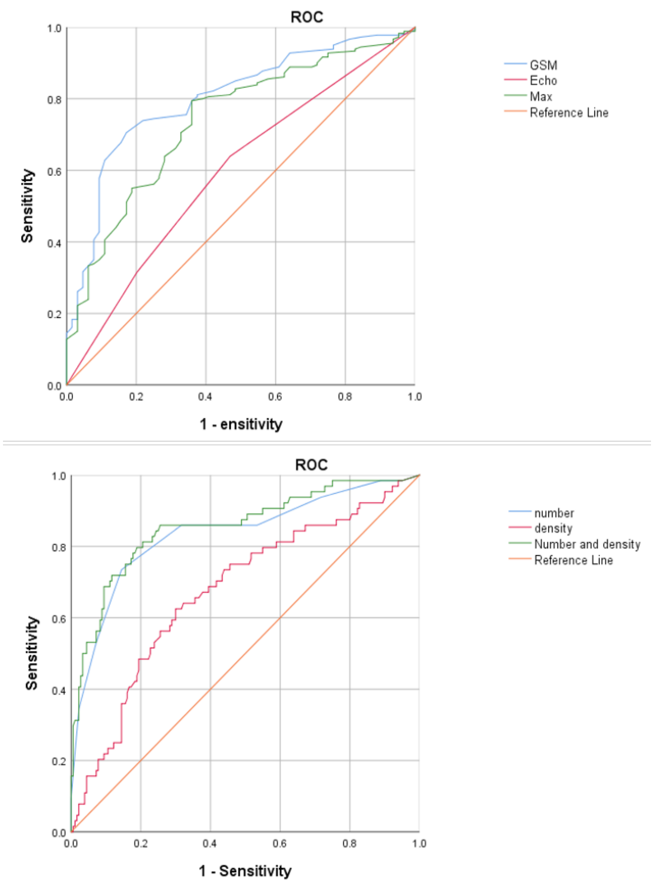


Figure 4: Diagnostic efficacy of various ultrasound techniques in determining plaque vulnerability.

Discussion

Recent studies [18] have shown that hypoechoic plaques have more distinct pathological features of vulnerable plaques than other echogenic plaques i.e., larger lipid core, thin fibrous cap, large number of inflammatory cells and neovascularization, which are more prone to intraplaque hemorrhage, rupture, etc., leading to the development and progression of acute stroke. This study indicated a significant correlation between both ultrasound echo type, median grayscale and plaque vulnerability. In many previous studies [19,20] visual classification was used to assess plaque echo, but the results were highly dependent on the operation and the subjective evaluation of the operator, so the use of computer-assisted plaque characterization methods can provide predictive clinical measurements [3], and the diagnostic sensitivity and specificity of plaque echo type for plaque vulnerability is lower than that of the median gray scale, so the application of the median gray scale for plaque Echoes are more accurately analyzed, and previous studies have demonstrated the relationship between median grayscale values and histopathology, with higher GSM values correlating with higher calcium percentages and fiber content [21], lower GSM values correlating with larger lipid cores [3], and lower GSM values on the plaque surface correlating with vulnerable plaques, similar to the above study, which demonstrated that lower GSM values of plaques are more prone to vulnerable plaques compared to higher GSM values, and this study also analyzed the previously unexplored association between plaque gray-scale features and plaque stiffness and neovascularization, as well as the value of assessing plaque stability. The results of this study suggest that the addition of median grayscale, ultrasound vascular and elasticity values, which are quantitative analysis metrics, to carotid ultrasound imaging may provide additional information to provide a prophylactic and therapeutic reference for assessing clinical or asymptomatic stroke risk and progressive cognitive decline.

The susceptibility of carotid plaques is related to mechanical properties [22], Shear Wave Elastography (SWE) has been shown to provide information about plaque stiffness, and lower Young's Modulus (YM) is associated with susceptible plaques. The purpose of this study was to determine a correlation between ultrasonic gray-scale plaque characteristics and plaque stiffness, as together they provide information on plaque composition. As found in this study, the YM values of plaques measured by SWE correlated with the plaque classification of different echoes, in agreement with the study of Ramnarine et al [23], and the mean YM range was compared as type I < type II < type III, with statistically significant differences between the maximum, intermediate and minimum YM in a two-by-two comparison, indicating that all three elasticity indices in the SWE technique can reflect the difference in this echo classification method between Therefore, in the future, using the range of YM values in this study as a tentative threshold for quantitative evaluation of plaque echo and prediction of vulnerability would be an ideal method to validate the results of this study. However, in a previous study, it was mentioned that in healthy carotid arteries, YM changes throughout the cardiac cycle [24], and whether YM values of carotid plaques show similar changes throughout the cardiac cycle and whether the cause of such changes is influenced by the intraplaque composition or by the action of the artery on plaque stretching during the cardiac cycle remains to be investigated further. In addition, the study has some limitations, on the one hand, the clinical evaluation of plaque echo is usually performed on two-dimensional images, but only a certain cross-section of the plaque is available, so these measurements are susceptible to changes due to the probe, the patient's movements, and the three-dimensional probe can be applied to observe and measure the volume of the plaque in all directions to reduce the occurrence of errors; on the other hand, the SWE technique used in this study On the other hand, the Q-box in the SWE technique used in this study is circular and cannot envelop the shape of the whole plaque, so multiple Q-boxes can only be used to cover the whole plaque as much as possible to evaluate the Young's modulus of the plaque as a whole, so there is a certain error, and it is also affected by the operator's technique.

In addition, the vulnerability of the plaque also depends on the presence and degree of formation of intraplaque neovascularization, which is also an important cause of intraplaque hemorrhage [25], and previous findings on the evaluation of carotid plaque morphology on 2D ultrasound and the ultrasound data from this study suggest that the use of 2D ultrasound alone to assess plaque echogenic characteristics and classify plaques to identify "high-risk "The GSM technique is not able to visualize the neovascularization within the plaque. CEUS has become a research hotspot for detecting intraplaque neovascularization in recent years, which is based on two-dimensional using contrast agent to enhance the backscattered echo, thus can more clearly display the arterial lumen and the surface condition of plaque, etc., this study analyzed the grading by the location of neovascularization and found that the plaques with different echogenic types were not statistically significant with the grading, and also analyzed the number and density of neovascularization and found that there were significant differences, which is consistent with the conclusion of Chaolun Li et al [26] that the lower the plaque echogenicity the more significant the enhancement, and the GSM In this study, we also found that the number and density of neovascularization were higher in the vulnerable plaque group, probably because [27] neovascularization is composed of a single layer of endothelial cells, which is prone to rupture and bleeding, leading to secondary thrombosis. The high permeability of neovascularization may also lead to acute ischemic stroke, which can easily infiltrate plasma inflammatory components and toxic substances into the extracellular media of the intima and media, reducing the oxygen diffusion capacity of the vessel wall and increasing the vulnerability of the plaque.

In this study, the diagnostic efficacy of each technique was compared, and the results showed that the sensitivity and specificity of echo type in the diagnosis of plaque vulnerability were poor, while median grayscale, ultrasound elasticity and ultrasonography imaging had higher diagnostic efficacy. In conclusion, gray-scale median, CEUS, and SWE are new ultrasound techniques that can be used to evaluate different echoes of plaque and determine vulnerability, and they can be used to comprehensively evaluate carotid plaque from multiple perspectives, which has positive guiding significance for the determination of vulnerable plaque and the prevention and treatment of cardiovascular and cerebrovascular diseases such as acute stroke.

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