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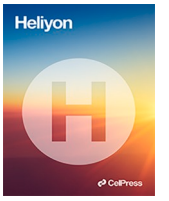

Research article

The value of contrast-enhanced ultrasonography in predicting stroke occurrence: A prospective study

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ABSTRACT

Objectives: This study aimed to investigate the association between contrast-enhanced ultrasound-detected (CEUS) perfusion patterns of carotid plaque and the occurrence of stroke.
Materials and methods: This prospective observational study finally enrolled 256 patients (151 of them having undergone CEUS) from 7851 patients who underwent carotid artery ultrasound from May 2019 to December 2019 with the endpoint being the occurrence of stroke. The risk factors and carotid ultrasound fetures was obtained from those patients. Analyze the relationship between these variables and stoke occurrence.
Results: Patients in the recurrent stroke group and those in the no-recurrent stroke group were statistically different in stenosis rate, plaque echo, fibrous cap integrity, calcification of fibrous cap, hypoechoic area within the plaque, and the pattern of neovascularization perfusion from plaque surface to plaque interior ($P < 0.05$). Upon adjusting for variables, in all subjects, Cox regression analysis showed that symptoms experienced within the past 6 months (RR = 2.486, 95 % CI: 1.282–4.821), moderate-to-severe carotid stenosis (RR = 2.407, 95 % CI: 1.480–4.593), calcification of fibrous cap (RR = 1.599, 95 % CI: 0.727–3.516) and patchy hypoechoic areas within plaque (RR = 2.486, 95 % CI: 1.107–5.578)independently predicted stroke occurrence across all subjects.; Among subjects underwent CEUS, Cox regression analysis demonstrated that moderate-to-severe carotid artery stenosis (RR = 2.105, 95 % CI: 1.425–4.510) and microbubbles entering the interior of the plaque from its surface (RR = 2.323, 95 % CI: 1.175–4. 594) were independent predictors of stroke occurrence.
Conclusions: The neovascularization perfusion pattern from the plaque surface to its interior serves as an independent predictor of stroke occurrence, thereby potentially enhancing clinical decision-making.

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Nonstandard abbreviations and acronyms

IPH	Intraplaque hemorrhage
LRNC	lipid-rich necrotic core
TRFC	Thin or ruptured fibrous cap
IPN	Intraplaque neovascularization
CT	Computed Tomography
MR	Magnetic resonance imaging
PET	Positron emission tomography
DSA	digital subtraction angiography
CEUS	Contrast-enhanced ultrasound
HR-MRI	High-resolution magnetic resonance imaging
IVUS	Intravascular ultrasound
ESVS	European Society for Vascular Surgery
CU	Conventional ultrasound
CCDUS	Cardiovascular and Cerebrovascular Disease Risk Assessment and Ultrasound Screening Promotion
TIA	Transient ischemic attack
NASCET	North American Symptomatic Carotid Endarterectomy Trial
NVE	New vascular event
REACH	REduction of Atherothrombosis for Continued Health
IMT	Intima-media thickness
ICC	Intraclass Correlation Coefficient
SD	Standard deviation
IQR	Interquartile range
HR	Hazard ratios
CI	Confidence interval

1. Introduction

Carotid artery stenosis is recognized as a significant contributor to ischemic stroke. The standard clinical treatment typically involves preventive medication, while individuals with moderate to severe stenosis may necessitate carotid artery revascularization procedures such as carotid endarterectomy or stenting, aim at preventing the embolization of plaques from the carotid artery and subsequent occlusion of distal arteries [1]. In recent decades, revascularization treatment strategies for ischemic stroke have primarily focused on the extent of carotid artery stenosis [2]. However, with the continuous advancement of medical imaging technology, an increasing number of studies suggest that assessing carotid atherosclerosis solely based on the degree of stenosis is insufficient. The stability and vulnerability of plaques are considered to be more crucial factors than the degree of stenosis [3].

The rupture of carotid atherosclerotic plaques can result in the embolization of plaque and thrombus to distal arteries, leading to ischemic stroke. Plaques that are more prone to rupture and are associated with a higher risk of ischemic stroke are referred to as vulnerable plaques. Vulnerable plaques exhibit histological characteristics that differ from stable plaques, including intraplaque hemorrhage (IPH), a lipid-rich necrotic core (LRNC), a thin or ruptured fibrous cap (TRFC), the presence of inflammatory cells, ulceration, calcification within the plaque, and intraplaque neovascularization (IPN) [4–7]. Numerous imaging techniques have undergone attempts and tests for the identification of vulnerable plaques. Computed Tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), digital subtraction angiography (DSA) and contrast-enhanced ultrasound(CEUS) can all serve as methods for the detection of vulnerability in extracranial carotid artery plaques [8]. However, high-resolution magnetic resonance imaging (HR-MRI) and intravascular ultrasound (IVUS) examinations are not suitable as first-line diagnostic methods for carotid artery stenosis as high cost and time-consuming nature for former, invasive, high radiation exposure, and limited mobility nature for latter. Furthermore, most vulnerable plaque features in extracranial carotid arteries are simply extrapolated from coronary artery plaques, and many studies infer their correlation with clinical events based on the morphological characteristics of the plaques. A notable limitation of this research field is the lack of prospective studies specifically targeting high-risk populations.

The ideal imaging modality for diagnosing stenosis would be one that minimizes risks, preferably a non-invasive method that allows for reproducibility and demonstrates histopathological correlation with plaque features. Moreover, it should be proven to be capable of predicting clinical outcomes [9]. Although ultrasound is operator-dependent, the guidelines provided by the European Society for Vascular Surgery (ESVS) state that color Doppler ultrasound is a B-Class recommended imaging method for carotid artery due to its non-invasive nature, highly reproducibleility, and ability to provide clear information about the carotid artery wall. If standardized training and continuous quality control are implemented for this examination, it has the potential to serve as a valuable diagnostic tool [10]. As a new dimension, CEUS could offer the capability to identify neovascularization within plaques, and these have been supported by a significant body of literature [11]. Our preliminary research indicates a correlation between perfusion patterns of carotid artery plaques and cerebrovascular events. Based on the characteristic features obtained from conventional ultrasound and CEUS of carotid plaques, we have established an initial diagnostic model [12,13]. However, the model has limited factors for diagnose,

only including ultrasound features, and the prospective validation is lacked. It is possible to identify patients with high-risk of stroke more accurately and thereby select treatment plans more effectively if prospective studies can confirm that the predictive factors of stroke can be obtained through the combination of risk factors and convenient non-invasive examinations.

Therefore, this study aims to extract features of plaque in conventional ultrasound(CU) and CEUS, prospectively investigate the relationship between plaque features and the occurrence/reoccurrence of stroke.

2. Methods

2.1. Study population

Between May 2019 and December 2019, participants were recruited from a population (n = 7851) referred for carotid ultrasound examination due to ischemic stroke/transient ischemic attack (TIA), carotid artery plaque, dizziness/transient blindness, and the presence of multiple cardiovascular risk factors. Participants without carotid artery plaque, those who had previously received neck radiation therapy or ipsilateral carotid revascularization surgery (including endarterectomy and stent implantation), those who refused to participate/did not complete the questionnaire and poor image quality (due to obesity, extreme distortion of vascular alignment, severe calcification and so on) were excluded. A total of 385 individuals were initially screened as study subjects (187 of them underwent CEUS). Subjects with one or more clinical symptoms within the past 6 months, including TIA, transient blindness, and mild or non-disabling stroke caused by ipsilateral intracranial vascular disease, were included in the symptomatic group (n = 147), 108 of them underwent CEUS. The asymptomatic group consisted of 238 individuals, 79 of them underwent CEUS. Patients in the symptomatic group underwent DSA/CTA examination within two weeks of enrollment. Based on the TOAST classification [14], those with cardioaortic embolism (n = 5), small-artery disease (n = 30), undetermined causes (n = 21), or unclassified stroke (n = 3) were excluded. Only patients with large-artery atherosclerosis stroke who had carotid artery stenosis $\geq$50 % or stenosis $\leq$50 % but with a suspicious vulnerable plaque detected by ultrasound/CTA/DSA (n = 88) were included. Then, totally 326 patients (symptomatic group: n = 88, 78 of them underwent CEUS; asymptomatic group: n = 238, 79 of them underwent CEUS) were follow up. After the follow up, 70 were lost(69 of asymptomatic group; 1 of symptomatic group). Finally, 256 patients (symptomatic group: n = 87, 77 of them underwent CEUS; asymptomatic group: n = 169, 74 of them underwent CEUS) were enrolled for subsequent analysis. The patient recruitment process is illustrated in Fig. 1. All patients gave written informed consent to participate, and all study protocols were performed in accordance with the 1975 version of the Declaration of Helsinki.

2.2. Clinical variables

The following clinical characteristics were collected through questionnaires and electronic medical records: (1) gender and age; (2) body mass index, hypertension, diabetes, coronary artery disease, dyslipidemia, personal history of stroke, smoking, alcohol consumption, and previous drug treatment.

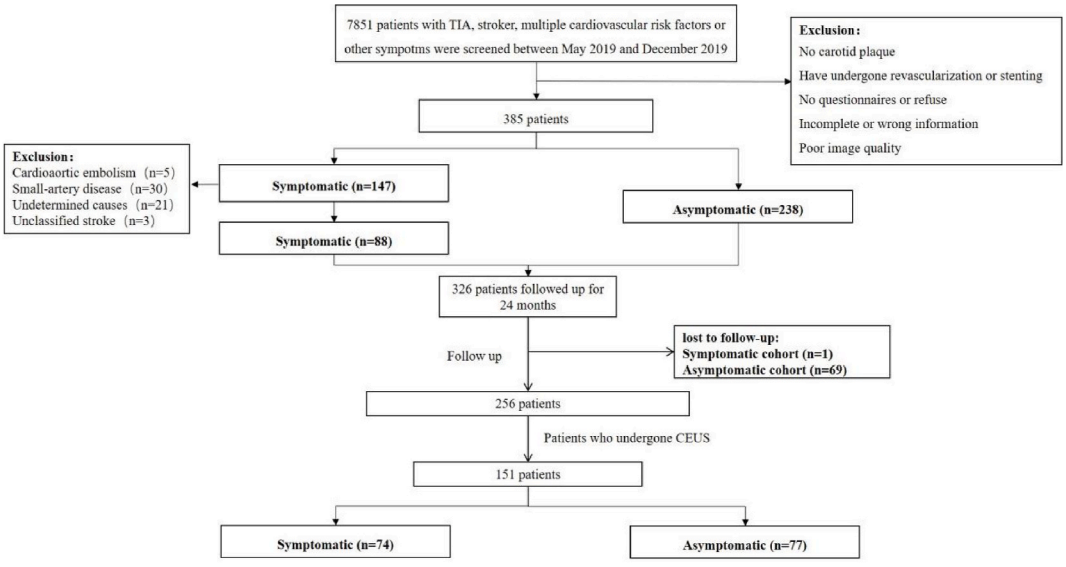

Fig. 1. Participant selection and follow-up. This flowchart shows the patient selection process, finally 256 patients were included in this study.

2.3. Follow-up

For each patient, follow-up was conducted every 6 months, with a maximum follow-up period of 24 months for each study participant. The primary outcome of the follow-up was new ischemic stroke/recurrent stroke. Ischemic stroke was defined as a new onset of persistent or transient neurologic deficits assessed by a neurologist.

2.4. Carotid ultrasound CEUS

Ultrasound instruments: (1) Aplio i900, 4–11 MHz L-probe, Canon Medical Systems, Tochigi, Japan. (2) SIEMENS ACUSON OXANA 2, 8-MHz Linear Probe (9L4), Erlangen, Germany. (3) Mindray Resona R9 3–9 MHz linear probe (L9-3u), Shenzhen, China.

To detect atherosclerotic lesions and assess plaque thickness, transverse and longitudinal views recorded from the proximal to the common carotid artery, above the clavicle, and extending to the bifurcation of the common carotid artery. Subsequently, the internal carotid artery was tracked as far as possible, maintaining a perpendicular angle to the neck. The entire bilateral common carotid arteries, internal and external carotid arteries, as well as the bifurcation and distal segments were examined. During this examination, we observed the three-layer structure of the main trunk and branches, assessed the intravascular lumen echo, and determined the presence of plaques through longitudinal sections. For identified plaques, the thickness was measured on the view showing the thickest cross-section of the plaque. For patients with multiple plaques, only the thickest plaque was observed and recorded for subsequent analysis. Carotid plaque is characterized by a focal region with a thickness of at least 1.5 mm, measured from the interface between the media and adventitia to the interface between the lumen and intima [15]. Alternatively, plaque can also be identified by the presence of focal wall thickening that is at least 50 % greater than the thickness of the surrounding vessel wall.

Afterwards, patients underwent CEUS. The contrast agent (sulfur hexafluoride microbubbles, Bracco Altana Pharma, Germany) was prepared by diluting the powder in 5 mL of physiological saline. Subsequently, 1.2 ml of the contrast agent was injected into the patient's median cubital vein, followed by the injection of 5 mL of physiological saline. Video recording began immediately after the injection of the 5 ml of physiological saline and continued for 150–180 s, with the image maintained in a plane longitudinally displaying the target plaque.

2.5. Image analysis and consistency evaluation

Static images and videos were interpreted by three different radiologists with over 10 years' experience in vascular ultrasound, who were blinded to the patients' medical histories. When there were discrepancies in observations, a consensus was reached after discussion. The intra-observer consistency was assessed by a senior vascular ultrasound expert with 13 years of experience. The inter-observer consistency and intra-observer consistency can be seen in Table A1.

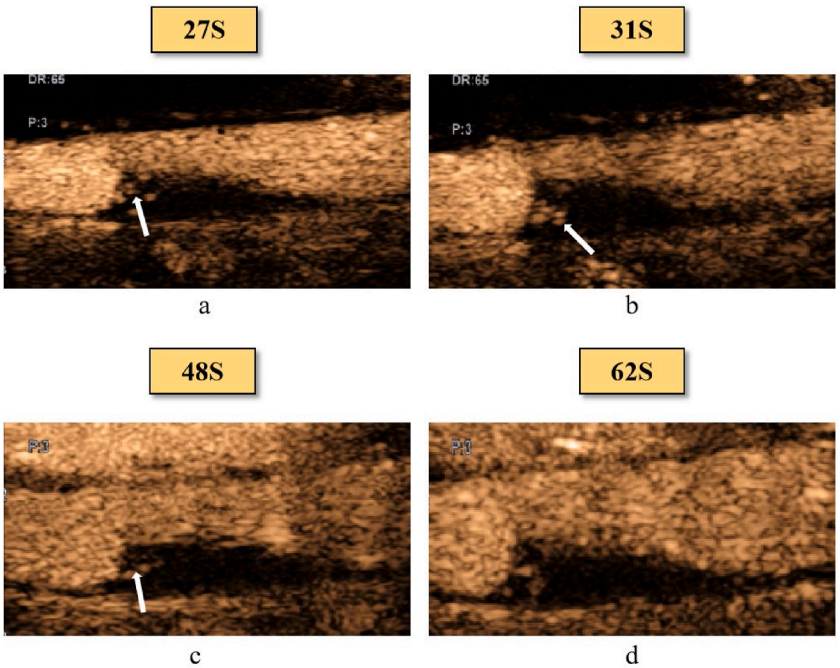

Fig. 2. a-d show the CEUS images at 27th, 31st, 48th and 62nd seconds, respectively. In contrast mode, microbubbles (white arrow) enter the interior of the plaque from an ulcer on the surface of the plaque.
Video1 demonstrated the dynamic contrast enhancement process of Fig. 2.

2.6. Ultrasounic characteristics

The definition of plaque characteristics remained consistent with our previous studies [12,13]. Plaque characteristics included those observed by conventional ultrasound and those observed by CEUS. (1)Conventional ultrasound characteristics: (I) plaque length and thickness; (II) plaque echo:hypoechoic (intraplaque echoes below the carotid intima-media layer),mixed echo (more than 20 % of the echoes within the plaque being discordant), hyperechoic (intraplaque echoes equal to or slightly above carotid artery outer layer of the carotid artery), plaque shape (regular or irregular), intraplaque hypoechoic areas; (III) intact fibrous cap (plaque surface is continuous and smooth, with no visible breaks, depressions, etc); (IV) fibrous cap calcification (punctate hyperechoic surface of plaque); (V)intraplaque calcification. (2) CEUS characteristics: (I)neovascularization: a semi-quantitative method was used to classify the degree of ultrasound enhancement within the plaque to assess neovascularization, which was divided into four levels: I to IV. Patients with I-II levels were considered to have less neovascularization, while patients with III-IV levels were considered to have more neovascularization; (II) the path of contrast agent entry into the plaque: this referred to the mode of microbubble entry from the bottom of the plaque (plaque surface entering mode, as shown in Fig. 2 and Video 1) or from the surface of the plaque (plaque entering mode, as shown in Fig. 3 and Video 2).

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2.7. Statistical analysis

Statistical analyses were performed with software (SPSS, version 21.0; IBM SPSS Statistics, Armonk, NY). The Kolmogorov–Smirnov test was performed to assess the normality of the data. Data that followed a normal distribution were presented as mean ± standard deviation (SD), while data that did not follow a normal distribution were described as median (interquartile range). Count data were presented as number (percentage). Student's t-tests for continuous variables and chi-square tests for dichotomous variables were adopted to identify the differences of population characteristics in different groups. Cox-regression models were adopted to examined the association between characters of plaque in ultrasound and NVE, and hazard ratios (HR) and 95 % confidence interval (CI) were calculated. The inter-observer reliability was assessed using the intraclass correlation coefficient (ICC). A two-sided $P < 0.05$ was considered to indicate trend. Numerous potential factors influence the occurrence of an outcome event, such as lifestyle, dietary habits, and medication use, so a detailed questionnaire was designed to collect this information and to include these factors as covariates.

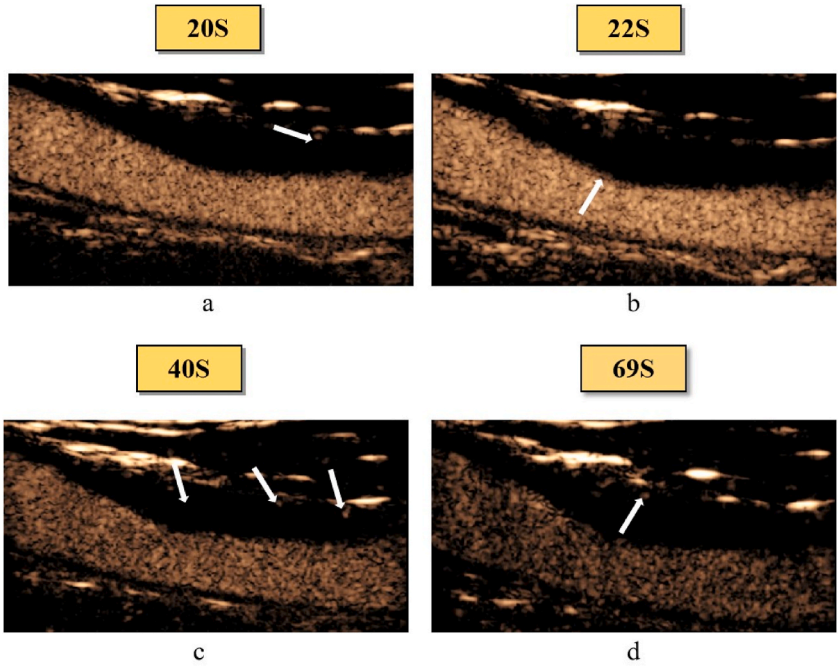

Fig. 3. a-d show the CEUS images at 20th, 22nd, 40th and 69th seconds, respectively. In contrast mode, microbubbles (white arrow) enter the interior of the plaque from the basement of the plaque.
Video 2 demonstrated the dynamic contrast enhancement process of Fig. 3.

3. Results

3.1. Participant characteristics

Among the 256 patients, the median follow-up time was 24 months (IQR 20–24), with a maximum follow-up time of 24 months. The symptomatic patient group consisted of 87 cases (1 lost), with a median age of 69 years (IQR 63–81, maximum age 85 years). The asymptomatic patient cohort included 169 cases (69 lost), with a median age of 67 years (IQR 61–73, maximum age 85 years). In all patients, 186 patients were male (72.66 %), and 78 of them (30.47 %) belonged to the symptomatic group while 108 (42.19 %) belonged to the asymptomatic group($P < 0.001$). 103 patients had hyperlipidemia (40.23 %), with 25 cases (9.77 %) in the symptomatic group and 78 cases (30.47 %) in the asymptomatic group($P = 0.007$). There were no significant statistical differences ($P > 0.05$) between the two groups in terms of risk factors such as hypertension, diabetes, stroke of personal history, stroke of family history, atrial fibrillation, and physical inactivity (Table 1).

The plaque thickness in the symptomatic group was 3.298 ± 1.157 mm, with a stenosis rate of 42.862 ± 18.766 %; in the asymptomatic group, the thickness was 2.631 ± 0.974 mm, with a stenosis rate of 30.195 ± 15.517 %, $P < 0.001$. In the symptomatic group, there were more plaques with characteristics observed in CU such as mixed echo, incomplete fibrous cap, and uneven plaque echogenicity than in asymptomatic group ($P < 0.05$). There were no significant statistical differences ($P > 0.05$) between the two groups regarding features such as the pattern of neovascularization perfusing from the plaque surface to the interior and the grade III-IV neovascularization within the plaque. The occurrence rate of stroke in the symptomatic group was significantly higher ($P = 0.004$) compared to the asymptomatic group, 29 cases (33.33 %) and 15 cases (8.88 %) respectively. Among the patients stroke, there were several notable characteristics observed in the plaques: moderate or severe stenosis, low echogenicity, incomplete fibrous cap, calcification of plaques, hypoechoic area within the plaques, and pattern of neovascularization from the plaque surface to the plaque interior ($P < 0.05$). However, there were no statistically significant differences between the stroke group and the no-stroke group in irregular plaque morphology and neovascularization grade III-IV within the plaque ($P > 0.05$), as shown in Table 2.

Cox regression analyses were performed separately on the entire study population (n = 256) and on subjects who underwent ultrasound imaging (n = 151) to identify risk factors associated with stroke occurrence. Table 3 presents the results of the entire study population, after adjusting for factors with $P < 0.05$, presence of symptoms within the past six months (HR = 2.486, $P = 0.007$), moderate-to-severe carotid artery stenosis (HR = 2.407, $p < 0.001$), fibrous cap calcification (HR = 1.599, $P = 0.015$), and patchy hypoechoic areas within plaque (HR = 2.486, $P = 0.020$) were significantly and independently associated with the occurrence or stroke. Table 4 presents the results for the subjects underwent CEUS. After adjusting for age, presence of symptoms within the past six months, plaque thickness, plaque echogenicity, and presence of calcification within the plaque, moderate-to-severe stenosis (HR = 2.105, CI: 1.425–4.580, $P = 0.037$) and the pattern of neovascularization perfusing from the plaque surface into the plaque (HR = 2.323, CI: 1.175–4.594, $P = 0.012$) were identified as significant and independent predictors of stroke. The KM survival curve demonstrates that patients with contrast agent entering from the bottom of the plaque exhibit a better disease-free survival rate compared to patients with contrast agent entering from the surface (Fig. 4).

Table 1
General characteristics of the study population.

Variables		Asymptomatic group (n = 169)	Symptomatic group (n = 87)	Total	P -value
Age,years, median (IQR)		67(61–73)	69(63–81)	256	<0.001
BMI, kg/m2, mean (IQR)		24(22–26)	23(21–26)	256	0.088
Current smoking	No	132	42	174	<0.001
	Yes	37	45	82	
Gender	Female	61	9	70	<0.001
	Male	108	78	186	
Hypertension	No	59	27	86	0.578
	Yes	110	60	170	
Diabetes	No	124	68	192	0.448
	Yes	45	19	64	
Stroke of family history	No	119	67	186	0.301
	Yes	50	20	70	
Previous stroke	No	124	61	185	0.659
	Yes	45	26	71	
Atrial fibrillation	No	163	84	247	1
	Yes	6	3	9	
Dyslipidemia	No	91	62	153	0.007
	Yes	78	25	103	

In this table, all study participants were categorized into symptomatic and asymptomatic groups to show their baseline characteristics separately. IQR indicates Interquartile range.

Table 2
Characteristics of plaque in CU and CEUS.

Character of plaque in CU		Asymptomatic (n = 169)	Symptomatic (n = 87)	Total	*P* -value	No Stroke (n = 212)	Stroke (n = 44)	Total	*P* -value
IMT		1.067 ± 0.227	1.082 ± 0.243		0.653	1.062 ± 0.230	1.116 ± 0.239		0.138
Plaque thickness		2.631 ± 0.974	3.298 ± 1.157		<0.001	2.730 ± 0.979	3.307 ± 1.276		0.002
Stenosis rate		30 ± 16	43 ± 19		<0.001	33 ± 17	42 ± 19		0.004
Hypoecho	No	86	43	129	0.895	96	33	129	0.018
	Yes	83	44	127		110	17	127	
Hyperecho	No	126	78	204	0.005	166	38	204	0.304
	Yes	43	9	52		46	6	52	
Mixed echo	No	126	53	179	0.031	153	26	179	0.003
	Yes	43	34	77		53	24	77	
Regular shape	No	123	53	176	0.064	147	29	176	0.088
	Yes	46	34	80		59	21	80	
Fibrous cap integrity	No	153	69	222	0.019	184	38	222	0.019
	Yes	16	18	34		22	12	34	
Calcification of fibrous cap	No	86	47	133	0.693	117	16	133	0.002
	Yes	83	40	123		89	34	123	
Echo uniformity	No	122	80	202	<0.001	159	43	202	0.245
	Yes	47	7	54		47	7	54	
Patchy hypoechoic areas within plaque	No	164	80	244	0.114	201	43	244	0.003
	Yes	5	7	12		5	7	12	
Calcification in plaque	No	42	32	74	0.058	67	7	74	0.009
	Yes	127	55	182		139	43	182	
Character of plaque in CEUS		Asymptomatic (n = 74)	Symptomatic (n = 77)	Total	*P* -value	No Stroke (n = 115)	Stroke (n = 36)	Total	*P* -value
microbubbles entered the plaque from the surface	No	41	42	83	1	70	13	83	0.012
	Yes	33	35	68		45	23	68	
neovascularization ≥ III	No	63	58	121	0.156	94	27	121	0.473
	Yes	11	19	30		21	9	30	

In this table, all study subjects (n = 256) and those who underwent CEUS (n = 151) were categorized into symptomatic and asymptomatic groups, stroke and no stroke groups, conventional ultrasound characteristics and CEUS features of plaques in each group were demonstrated. CU indicates Conventional ultrasound. CEUS indicates Contrast-enhanced ultrasound. IMT indicates Intima-media thickness.

4. Discussion

The vulnerable plaque in the carotid artery is an important risk factor for the occurrence and recurrence of stroke [16,17]. The PARISK study in 2022 conducted a prospective study in patients with symptomatic mild to moderate carotid stenosis [18], and found that the presence of IPH was associated with recurrent ipsilateral ischemic stroke or TIA. HR-MRI is required to visualize the thin fibrous cap, while IPN is also considered a crucial feature. Previous studies [19,20] have demonstrated that neovascularization and IPH are necessary process in the transformation of stable plaques into unstable ones, ultimately leading to plaque rupture. Therefore, identifying the formation and corresponding characteristics of neovascularization within the plaque is of great clinical value for the identification of high-risk individuals, prevention of adverse events, and selection of treatment strategies.

CEUS has been demonstrated to enhance the diagnostic accuracy of detecting plaques and stenosis [21–23]. Compared to conventional two-dimensional ultrasound, contrast agents improve the detection rate of plaques, such as local perfusion defects are more easily noticed than hypoechoic plaques. Additionally, CEUS provides valuable information about intraplaque blood flow characteristics that cannot be obtained by B-mode ultrasound alone. When compared with reference methods such as CT, MRI, and histology, CEUS has shown good ability to characterize neovascularization within vulnerable plaques. Previous studies have typically focused on whether plaques show enhancement and the intensity of enhancement, which reflects the number and density of neovessels within the plaque. However, in our previous retrospective study [12], we found that in addition to the level of plaque enhancement, the pattern of perfusion (i.e., whether the contrast agent enters the plaque from the surface or the base) is associated with the degree of carotid artery stenosis. In patients with severe stenosis, the contrast agent tends to enter the plaque from the surface, suggesting potential infiltration of the contrast agent caused by plaque surface or thin fibrous cap rupture. Such perfusion patterns may indicate an impending plaque rupture. In this scenario, the plaque surface is susceptible to thrombus adhesion, further exacerbating the harm caused by the plaque. This not only increases the vulnerability of the plaque itself but also facilitates easy detachment of the thrombus, leading to occlusion of intracranial blood vessels under the impact of blood flow and ultimately resulting in severe cardiovascular and cerebrovascular events. Therefore, we designed a prospective study to investigate the relationship between enhancement characteristics of plaques and the occurrence of stroke. The results confirmed that the pattern of contrast agent perfusion, entering the plaque from the surface (RR = 2.323, 95%CI: 1.175–4.594, $P = 0.012$), was an independent risk factor associated with the occurrence or recurrence of stroke. The presence of such perfusion pattern in the plaque suggests a potential for future severe cerebrovascular events, Figs. 5–6 and Video 3–4 illustrates a typical case. However, there was no significant difference in the enhancement level of neovascularization between stroke and no stroke patients ($P = 0.473$). This probably due to the fact that the assessment of microbubble enhancement within the plaque is

Table 3
Risk factors for stroke in all participants.

Variable	Univariable Analysis			Multivariable Analysis		
	HR	95 % CI	*P*-value	Adjusted HR	95 % CI	*P*-value
Gender						
female	Ref			–	–	–
male	1.246	0.649–2.389	0.508			
Age	1.042	1.013–1.072	0.004	1.02	0.090–1.020	0.184
BMI	0.959	0.881–1.045	0.339	–	–	–
Smoking						
no	Ref					
yes	0.894	0.488–1.638	0.718	–	–	–
Hypertension						
no	Ref					
yes	1.944	0.996–3.797	0.051	–	–	–
Diabetes						
no	Ref					
yes	1.368	0.747–2.505	0.31	–	–	–
Stroke of family history						
no	Ref					
yes	0.817	0.427–1.563	0.541	–	–	–
Symptoms in the past 6 months						
no	Ref					
yes	3.072	1.752–5.388	0.001	2.486	1.282–4.821	0.007
Previous stroke						
no	Ref					
yes	1.69	0.949–3.014	0.0749	–	–	–
Atrial fibrillation						
no	Ref					
yes	2.543	0.791–8.175	0.117	–	–	–
Physical inactivity						
no	Ref					
yes	0.913	0.444–1.880	0.806	–	–	–
IMT	2.793	0.813–9.594	0.103	–	–	–
Plaque thickness	1.447	1.175–1.783	0.001	1.118	0.833–1.501	0.457
Stenosis						
<50 %	Ref					
≥50 %	2.537	1.446–4.449	0.001	2.407	1.480–4.593	0.001
Hyperechoic plaque						
no	Ref					
yes	0.948	0.461–1.952	0.885	–	–	–
Hypoecho plaque						
no	Ref					
yes	0.46	0.256–0.827	0.009	0.73	0.288–1.852	0.507
Mixed echogenic plaque						
no	Ref					
yes	2.324	1.335–4.049	0.003	0.656	0.255–1.685	0.381
Regularly shaped plaque						
no	Ref					
yes	1.532	0.874–2.687	0.137	–	–	–
Fibrous cap integrity						
no	Ref					
yes	2.015	1.053–3.856	0.034	0.978	0.480–1.990	0.95
Calcification of fibrous cap						
no	Ref					
yes	2.444	1.348–4.428	0.003	1.599	0.727–3.516	0.015
Patchy hypoechoic areas within plaque						
no	Ref					
yes	3.341	1.503–7.428	0.003	2.486	1.107–5.578	0.02
Calcification in plaque						
no	Ref					
yes	2.703	1.216–6.009	0.015	2.245	0.795–6.340	0.127

Association between clinical and carotid ultrasound features and stroke in all study population. HR indicates hazard ratio. CI indicates confidence interval. Ref indicates reference.

based on a visual evaluation method [24], which provides qualitative assessments of the presence or absence of microbubbles, and limited evaluations such as few, moderate, or extensive microbubbles. Such qualitative and subjective evaluation methods restrict the value of microbubble enhancement assessment in plaque vulnerability evaluation, and more complex methods such as built-in software packages and sophisticated algorithms [25] are required to achieve quantification. In addition, these results may also be influenced by the characteristics of the studied population. Some of our study population had symptoms within the past six months

Table 4
Risk factors for stroke in participants who underwent CEUS.

Variable	Univariable Analysis			Multivariable Analysis		
	HR	95 % CI	*P*-value	Adjusted HR	95 % CI	*P*-value
Sex						
female	Ref					
male	1.026	0.466–2.257	0.95	–	–	–
Age	1.049	1.016–1.083	0.003	1.043	1.010–1.077	0.13
BMI	0.924	0.832–1.027	0.144			
Smoking						
no	Ref					
yes	0.856	0.427–1.714	0.66	–	–	–
Hypertension						
no	Ref					
yes	1.408	0.661–2.999	0.376	–	–	–
Diabetes						
no	Ref					
yes	1.288	0.605–2.743	0.511	–	–	–
Stroke of family history						
no	Ref					
yes	0.436	0.169–1.125	0.086	–	–	–
Symptoms in the past 6 months	Ref					
no						
yes	2.472	1.186–5.150	0.016	1.882	0.869–4.080	0.109
Previous stroke						
no	Ref					
yes	1.808	0.910–3.593	0.091	–	–	–
Atrial fibrillation						
no	Ref					
yes	3.172	0.967–10.404	0.057	–	–	–
Physical inactivity						
no	Ref					
yes	0.985	0.382–2.541	0.975			
IMT	1.826	0.441–7.561	0.406	–	–	–
Plaque thickness	1.383	1.084–1.764	0.009	1.239	0.831–1.646	0.293
Stenosis						
<50 %	Ref					
≥50 %	2.363	1.229–4.545	0.01	2.105	1.425–4.510	0.037
Hyperechoic plaque						
no	Ref					
yes	0.822	0.251–2.691	0.746	–	–	–
Hypoecho plaque						
no	Ref					
yes	0.431	0.211–0.878	0.02	0.228	0.106–0.673	0.227
Mixed echogenic plaque						
no	Ref					
yes	2.412	1.230–4.729	0.01	1.387	0.620–3.100	0.426
Regularly shaped plaque						
no	Ref					
yes	1.586	0.823–3.054	0.168	–	–	–
Fibrous cap integrity						
no	Ref					
yes	1.777	0.855–3.689	0.123	–	–	–
Calcification of fibrous cap						
no	Ref					
yes	1.434	0.739–2.786	0.287	–	–	–
Patchy hypoechoic areas within plaque						
no	Ref					
yes	1.225	0.375–4.004	0.737	–	–	–
Calcification in plaque						
no	Ref					
yes	1.003	1.153–6.444	0.022	1.001	0.448–2.541	0.196
CEUS perfusion mode						
From the base	Ref					
From the surface	2.409	1.219–4.759	0.011	2.323	1.175–4. 594	0.012
Neovascularization						
1-II	Ref					
III-IV	1.386	0.651–2.951	0.396	–	–	–

Association between clinical and carotid ultrasound features and stroke in patients underwent CEUS. CEUS indicates Contrast-enhanced ultrasound. HR indicates hazard ratio. CI indicates confidence interval. Ref indicates reference.

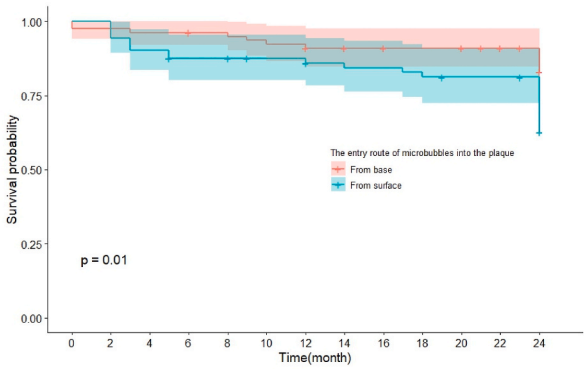

Fig. 4. Survival of patients underwent CEUS. There was a difference in survival between the two groups, patients with CEUS demonstrating microbubbles entering the plaque from the base have a higher event-free survival.

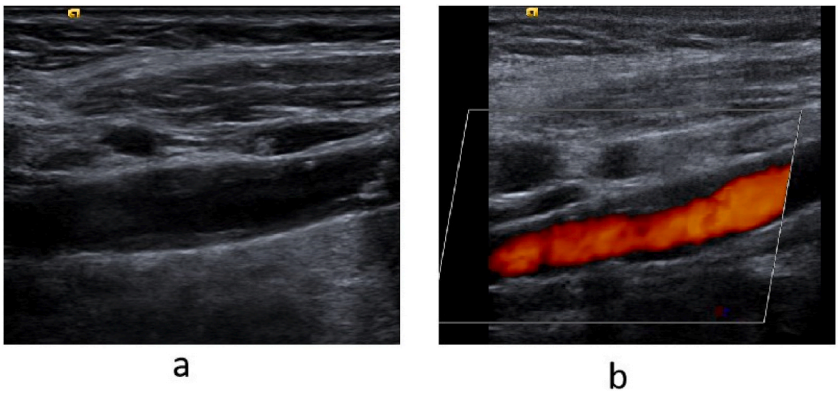

Fig. 5. June. 2019, a 77 years old female patient, with headache, nausea and vomiting for 1 day, CT showed multiple lacunar lesion in basal ganglia regions and bilateral frontoparietal lobes. a-b. Conventional ultrasound demonstrated a hypoechoic plaque in the upper segment of the right common carotid artery with a still regular morphology and an uneven fibrous cap, with no defects seen.

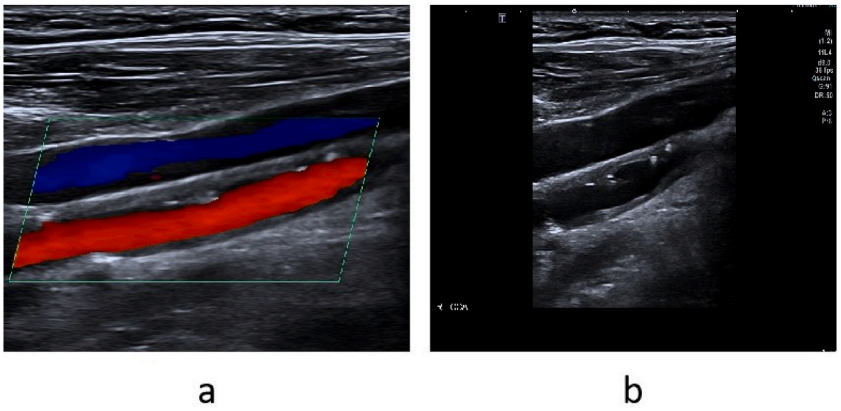

Fig. 6. June. 2021, the patient was hospitalized for subacute cerebral infarction. a-b. Conventional ultrasound demonstrated a hypoechoic plaque, defective fiber cap, strong punctate echoes on the cap and inside the plaque.

Video 3. June. 2019, a 77 years old female patient, with headache, nausea and vomiting for 1 day, CT showed multiple lacunar lesion in basal ganglia regions and bilateral frontoparietal lobes. a. The dynamic grayscale image of the plaque, in longitudinal section; b. The dynamic grayscale image of the plaque, cross-sectioned; c. In CEUS mode, the plaque body is seen to be perfused with large sheets of microbubbles, which enter the interior from the plaque surface.

Video 4. June. 2021, the patient was hospitalized for subacute cerebral infarction. Conventional ultrasound demonstrated a hypoechoic plaque, defective fiber cap, strong punctate echoes on the cap and inside the plaque; a. The dynamic grayscale image of the plaque, in longitudinal section; b. In CEUS mode, the plaque body is seen to be perfused with large sheets of microbubbles, which enter the interior from the plaque surface.

(87/256). For such individuals, the significance of perfusion pattern may be greater than perfusion intensity, which requires further investigation. The evaluation of perfusion pattern is less subjective, as it not only reflects neovascularization intensity but also potentially indicates structural changes such as minor disruptions on the plaque surface, which are considered precursors to plaque rupture, such as a thin fibrous cap. Therefore, CEUS has important application value in the future evaluation of plaque vulnerability, but at the same time, it is necessary to ensure that the evaluation criteria are as clear as possible to improve diagnostic consistency and accuracy.

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38621

In addition to the plaques, we also emphasize other features that contribute to the occurrence of cardiovascular events. We found that the presence of symptoms within the past six months (HR = 2.486, P = 0.007) and moderate or severe stenosis (HR = 2.407, P < 0.001) were independent predictors of stroke occurrence/recurrence, which HR were not lower than those of plaque characteristics. Carotid artery stenosis is well-known as a major cause of ischemic cerebrovascular disease. Atherosclerotic stenosis of the carotid artery leads to insufficient cerebral blood flow, which is a risk factor for cerebral ischemia and stroke. Currently, the treatment of carotid artery stenosis and indications for surgery are based on the NASCET criteria [26] and recommendations from the European Society for Vascular Surgery, which state that surgical intervention should be considered for stenosis greater than 70 % or greater than 50 % with other conditions present. However, recent studies have demonstrated that even patients with low degrees of stenosis have a significant risk of recurrent stroke. In this study, the stroke occurrence rate for patients without prior stroke was 33.3 %, and the stroke recurrence rate for patients with prior stroke was 8.88 %. The corresponding stenosis rates were 30.195 ± 15.517 % and 42.862 ± 18.766 %, respectively. The majority of patients did not reach stenosis rates of 50 % or 70 %, yet they still had a considerable risk of stroke occurrence/recurrence. This suggests that we need to reevaluate the treatment approach for these patients and improve risk stratification based on plaque characteristics and clinical features to identify high-risk patients. Early detection of the existence of vulnerable plaques can effectively prevent the majority of strokes and stroke recurrence, but the specific benefits need to be further confirmed by large-scale randomized controlled studies. In terms of imaging modalities, previous studies have generally considered MRI combined with MRA, DSA, and HR-MRI as effective methods for evaluating stenosis and plaque histological features. However, there are various issues in clinical practice, such as vascular remodeling, long examination times, patient discomfort due to long periods of immobilization and noise, and patient non-compliance, such as artifacts caused limb movement, and anxiety. By contrast, the examination time of CEUS is generally less than 10 min. We believe that CEUS can play a greater role in identifying high-risk patients and follow-up in high-risk patients.

As for generalizability, any explanation for an association between NVE and the pattern of neovascularization perfusion of plaque in CEUS must be conjectural. There is no published evidence to suggest a direct biologic mechanism, and no other epidemiologic studies of pathological study with relevant results. More evidence is needed due to the lack of corroborative evidence, especially the studies were based on relatively small populations and single center study. Therefore, rather than changing prescribing practice, we recommend it as a direction for the study of accurate imaging of vulnerable plaques.

This study is the first study to include contrast agent perfusion patterns, which were not included in previous studies. Additionally, it is a prospective clinical study, which allows for better observation and analysis of the relationship between the target features and outcomes. But there are also some limitations. Firstly, some subjects were loss to follow-up. Although we enrolled a representative population with some external generalizability, the single-center study design makes generalizing the findings limited. Certainly, this study provides a basis for multicenter study with a more multidimensional design. Compared to the initial number of enrolled patients, we had a close to 60 cases lost to follow-up and the follow-up period was only 2 years, mainly due to the impact of the pandemic of COVID-19. However, we conducted a detailed review of data during and after the study to ensure its reliability. Secondly, there were issues with medication usage, as the information provided was not detailed enough. Additionally, due to various reasons such as waiting for examinations and referrals, routine ultrasound, contrast-enhanced ultrasound, DSA/CTA could not be completed in a single day. However, we performed CTA/DSA examinations on all enrolled symptomatic patients, excluding small artery type and other types, to ensure that the plaques in symptomatic patients were responsible for the symptoms. Therefore, the relationship between plaque vulnerability and small artery occlusive stroke cannot be determined at this stage. We will conduct further research to improve risk assessment by incorporating vulnerable feature and include histological evidences in new studies. It should be noted that some features showed significance in both Cox regression analyses, but with different HR. Additionally, certain features only showed significance in Cox regression analysis in specific subgroups, which may be influenced by factors such as reduced sample size and subset-specific features. The smaller sample size may lead to less precise estimation of the risk ratio, and subset-specific features may influence the values of the risk ratio.

5. Conclusion

Clinical symptoms within the past six months, moderate or above carotid artery stenosis, fibrous cap calcification, and neovascularization pattern from plaque surface to plaque interior are independent predictive factors for stroke occurrence. The perfusion pattern of CEUS agent entering from the plaque surface indicates a higher risk of stroke occurrence. These results deepen our understanding of plaque vulnerability and contribute to improving clinical decision-making. However, further research is still needed to validate these findings and confirm the population that would benefit from them.

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Clinical trial registration information

This study is registered on the Chinese Clinical Trial Registry Platform (http://www.chictr.org.cn) under approval number ChiCTR 2000040163. The research protocol has been approved by the Institutional Review Board of Shanghai Tongren Hospital with the approval number SHTR 2020-085-01 in 23, Dec 2020.

Data availability statement

The datasets used during the current study available from the first author on reasonable request.

CRediT authorship contribution statement

Yunqian Huang: Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Chuanjian Chen:** Writing – original draft, Methodology, Formal analysis. **Junni Shi:** Investigation. **Yuqun Wang:** Investigation. **Yanchun Xie:** Investigation. **Lixia Zhang:** Investigation. **Wenqian Zhu:** Investigation. **Jiatong Xu:** Writing – original draft. **Man Chen:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Pinjing Hui:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yunqian Huang reports financial support was provided by Shanghai Municipal Health Commission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. table

Table A1
Consistency evaluation

	Inter-observer Agreement in assessment of carotid plaque ultrasound characteristics by three ultrasound physicians			Intra-observer Agreement of carotid plaque ultrasound characteristics by senior ultrasound physician at two different times		
	ICC	*P*-value	95 % CI	ICC	*P*-value	95 % CI
intima-media thickness	0.809	0.001	0.706–0.885	0.886	0.001	0.764–0.925
Carotid plaque thickness	0.957	0.001	0.929–0.975	0.966	0.001	0.938–0.982
Hypoecho	0.873	0.001	0.800–0.925	0.953	0.001	0.914–0.974
Hyperecho	0.729	0.001	0.597–0.832	0.848	0.001	0.735–0.915
Mixed echo	0.719	0.001	0.584–0.826	0.845	0.001	0.730–0.914
Shape	0.840	0.001	0.750–0.904	0.903	0.001	0.827–0.947
Integrity of fibrous cap	0.839	0.001	0.749–0.903	0.911	0.001	0.841–0.951
Calcification of fibrous cap	0.758	0.001	0.635–0.852	0.921	0.001	0.858–0.956
Echo uniformity	0.902	0.001	0.843–0.942	0.951	0.001	0.912–0.973
Patchy hypoechoic areas	0.810	0.001	0.709–0.885	0.934	0.001	0.880–0.964
Calcification in plaque	0.871	0.001	0.797–0.923	0.952	0.001	0.913–0.974
Plaque calcification score	0.895	0.001	0.833–0.938	0.916	0.001	0.848–0.954
Neovascularization	0.777	0.001	0.661–0.864	0.849	0.001	0.737–0.916
CEUS perfusion pattern	0.836	0.001	0.746–0.902	0.872	0.001	0.775–0.929

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