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Value of Intraplaque Neovascularization on Contrast-Enhanced Ultrasonography in Predicting Ischemic Stroke Recurrence in Patients With Carotid Atherosclerotic Plaque

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Objective: Patients with a history of ischemic stroke are at risk for a second ischemic stroke. This study aimed to investigate the relationship between carotid plaque enhancement on perfluorobutane microbubble contrast-enhanced ultrasonography (CEUS) and future recurrent stroke, and to determine whether plaque enhancement can contribute to risk assessment for recurrent stroke compared with the Essen Stroke Risk Score (ESRS).

Materials and Methods: This prospective study screened 151 patients with recent ischemic stroke and carotid atherosclerotic plaques at our hospital between August 2020 and December 2020. A total of 149 eligible patients underwent carotid CEUS, and 130 patients who were followed up for 15–27 months or until stroke recurrence were analyzed. Plaque enhancement on CEUS was investigated as a possible risk factor for stroke recurrence and as a possible adjunct to ESRS.

Results: During follow-up, 25 patients (19.2%) experienced recurrent stroke. Patients with plaque enhancement on CEUS had an increased risk of stroke recurrence events (22/73, 30.1%) compared to those without plaque enhancement (3/57, 5.3%), with an adjusted hazard ratio (HR) of 38.264 (95% confidence interval [CI]:14.975–97.767; $P < 0.001$) according to a multivariable Cox proportional hazards model analysis, indicating that the presence of carotid plaque enhancement was a significant independent predictor of recurrent stroke. When plaque enhancement was added to the ESRS, the HR for stroke recurrence in the high-risk group compared to that in the low-risk group (2.188; 95% CI, 0.025–3.388) was greater than that of the ESRS alone (1.706; 95% CI, 0.810–9.014). A net of 32.0% of the recurrence group was reclassified upward appropriately by the addition of plaque enhancement to the ESRS.

Conclusion: Carotid plaque enhancement was a significant and independent predictor of stroke recurrence in patients with ischemic stroke. Furthermore, the addition of plaque enhancement improved the risk stratification capability of the ESRS.

Keywords: Intraplaque neovascularization; Contrast-enhanced ultrasonography; Stroke recurrence; Carotid plaque; Prediction

INTRODUCTION

Stroke is the leading cause of disability and death in adults worldwide [1]. Approximately three-quarters of the global burden of stroke deaths occur in low- and middle-income countries, including China [2]. Patients with recent ischemic stroke are at risk of a second ischemic stroke, with 11% of individuals having a recurrence within 1 year of the first stroke and 26% within 5 years [3]. Secondary prevention measures reduce the risk of secondary ischemic stroke by 20%–30% [4]. Effectively identifying independent predictors of ischemic stroke recurrence and promptly enacting secondary preventive measures are expected to reduce the risk of stroke recurrence. The Essen Stroke Risk Score (ESRS) has been used to predict recurrent ischemic stroke in patients with no age restriction [5]. However, the evaluation

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criteria did not consider information on atherosclerotic plaques detected using noninvasive imaging technologies. Previous evidence has indicated that carotid artery stenosis is associated with an increased risk of recurrent stroke [6]. Although severe carotid stenosis can cause recurrent stroke, patients with nonsevere carotid stenosis may also experience recurrent stroke [7]. Intraplaque neovascularization (IPN) is a hallmark of vulnerable plaques that are most likely to rupture and precipitate ischemic stroke [8]. Contrast-enhanced ultrasonography (CEUS) can be used to visualize neovascularization within atherosclerotic plaques and identify vulnerable plaques [9]. Previous studies have demonstrated that IPNs, assessed using CEUS, are consistent with plaque neovascularization density detected by histology and immunohistochemistry [10,11]. Although several studies have shown that IPN is an independent predictor of stroke [12-14], the association between the carotid plaque enhancement on CEUS and stroke recurrence and its additional contribution to ESRS for the prediction of recurrent stroke is not fully understood [15,16]. Therefore, our study investigated the relationship between IPN, detected using perfluorobutane microbubbles-CEUS, within carotid plaques and future recurrent stroke and determine whether IPN can contribute to risk assessment for recurrent stroke compared with the ESRS.

MATERIALS AND METHODS

Patients

This prospective study was approved by the institutional review board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, and written informed consent was obtained from all participants before examination (IRB number TJ-IRB20210606). We screened 151 patients with a recent ischemic stroke and carotid atherosclerotic plaques at our hospital between August 2020 and December 2020. Ischemic stroke was defined as a focal neurological deficit lasting more than 24 h, with computed tomography and/or magnetic resonance imaging evidence of cerebral infarction. The inclusion criteria were ischemic stroke and at least one carotid atherosclerotic plaque (> 2.0 mm) located on the same side as the ischemic stroke, detected by conventional ultrasound examination. The exclusion criteria were cardioembolism, small-vessel occlusion, stroke of other determined etiology, stroke of undetermined etiology, previous carotid endarterectomy,

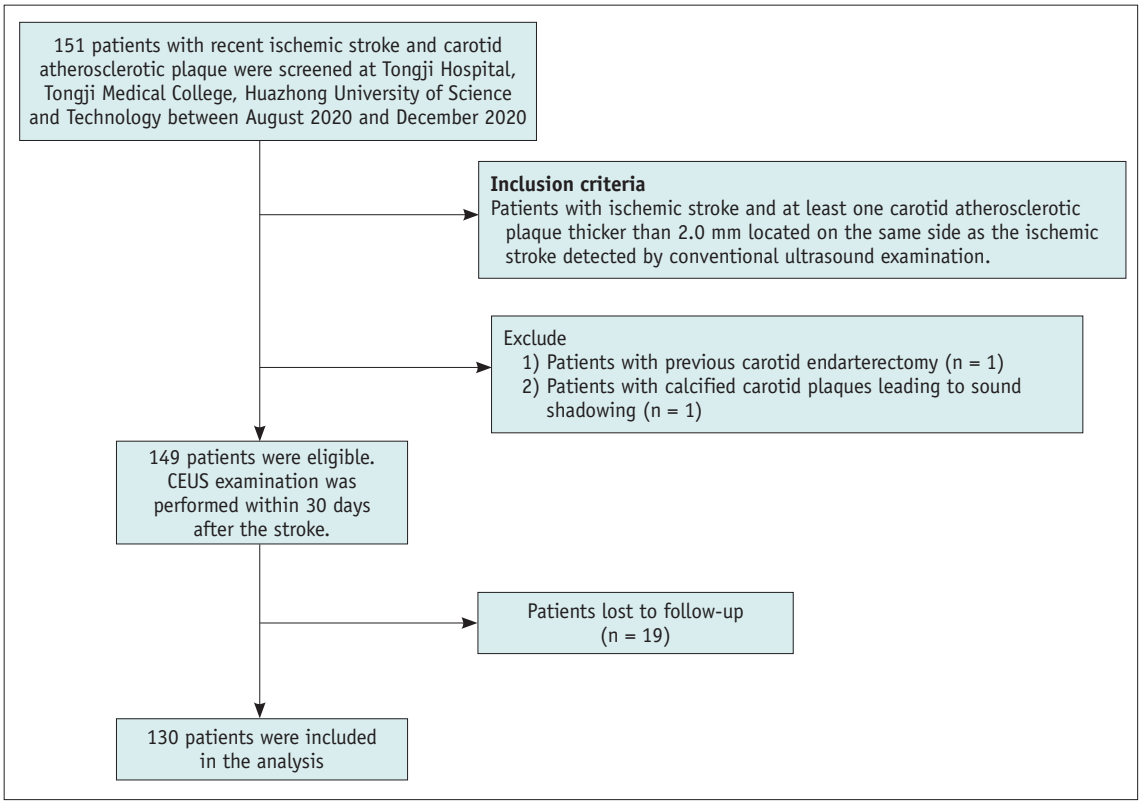

Fig. 1. Flow chart of patient recruitment. This flowchart shows the patient selection process, including the inclusion criteria, and finally 130 patients were included in this study. CEUS = contrast-enhanced ultrasonography

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and calcified carotid plaques leading to sound shadowing. After applying the inclusion and exclusion criteria, 149 patients were eligible and underwent CEUS (Fig. 1). CEUS examination was performed within 30 days after stroke.

Clinical Information

Data on clinical characteristics (sex, age, body mass index, smoking status, presence of hypertension, diabetes, and coronary heart disease) and laboratory test results (white blood cell count, high-sensitivity C-reactive protein, cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels) were collected from electronic medical records.

Standard Carotid Ultrasound and CEUS

Standard ultrasound and CEUS were performed within 1 week of patient admission using an ultrasound machine (GE Logiq 9, GE Healthcare) with a 9-L probe and a 6–8 MHz transmission frequency by one of the researchers who was blinded to participant history. This experienced radiologist had 30 years of clinical experience with standard carotid ultrasound and more than 10 years of clinical experience with CEUS. If a plaque was identified, the view showing the thickest cross-section of the plaque was used to measure the maximal carotid plaque thickness with electronic calipers. In patients with multiple plaques, only the thickest plaque was observed and recorded for analysis during standard carotid ultrasound and CEUS.

Conventional US features, including plaque thickness, length, burden, remodeling index, and eccentricity index, were measured and calculated from the images [17,18]. Plaques were characterized by their appearance on US images and classified as soft, hard, calcified, or mixed, according to the widely used criteria [19]. Plaque ulcer was defined as depression depth and width ≥ 2 mm, and irregular plaques were defined as those whose depression depth and width were both < 2 mm [20]. Carotid artery diameter stenosis was classified as mild (< 50%), moderate (50%–69%), or severe (≥ 70%) [21].

The patients then underwent CEUS examination, with special attention paid to previously identified plaques. To reduce the destruction of microbubbles, we preset a mechanical index of 0.24 and a frame rate of 12/s. A 1.0 mL of contrast agent Sonazoid (GE Healthcare) was injected over 2–3 s via the antecubital vein, followed by a bolus injection of 5–10 mL of normal saline. Images taken at least 3 s before and 5 min after the appearance of the contrast effect in the carotid lumen were acquired and recorded for subsequent

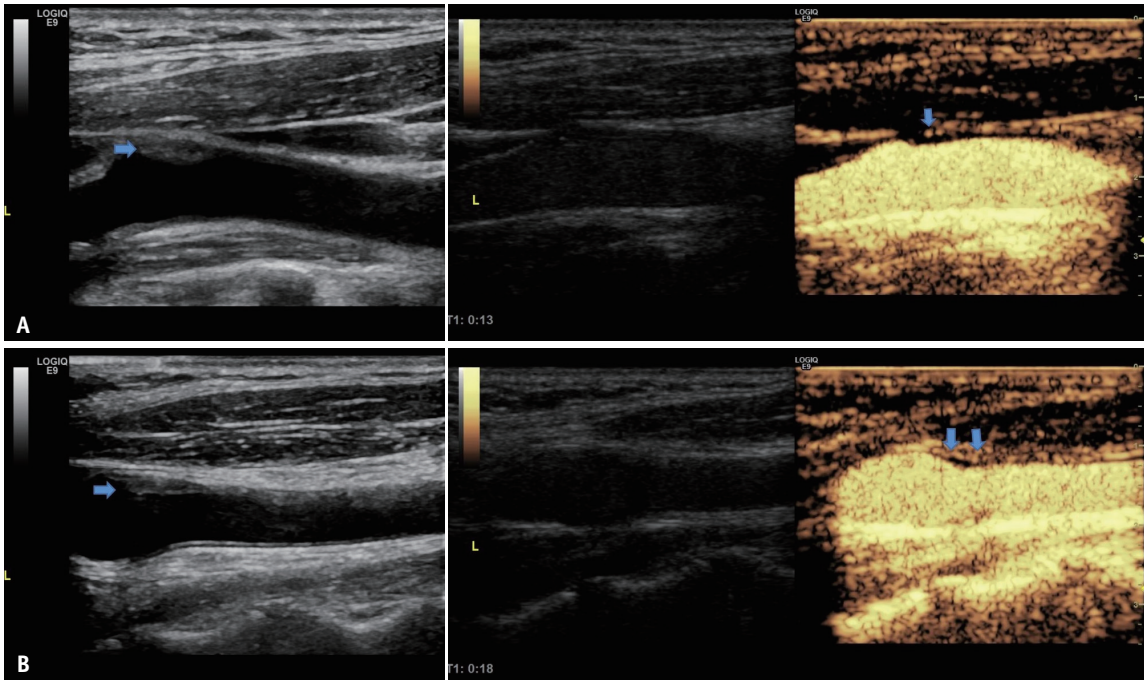

Fig. 2. Typical examples of conventional longitudinal carotid ultrasonographic images (left panel) and grades of carotid intraplaque neovascularization, based on contrast-enhanced ultrasonography examination (right panel). **A.** Grade 1: absence of enhancement in the core of plaque, and enhancement limited to the adventitia side of the plaque (arrows). **B.** Grade 2: extensive contrast enhancement throughout the plaque (arrows).

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analysis. The presence of plaque contrast enhancement was identified based on the dynamic movement of microbubble reflectors within the plaque. The CEUS examination results of carotid plaques were divided into two grades. Grade 1 indicated the absence of enhancement within the plaque or enhancement limited to the shoulder and adventitia side of the plaque, and grade 2 indicated enhancement of the plaque core or extensive contrast enhancement throughout the plaque (Fig. 2) [22].

ESRS and ESRS Plus CEUS

The ESRS includes the following variables: age 65–75 years, age ≥ 75 years (2 points), hypertension, diabetes mellitus, myocardial infarction, prior stroke or transient ischemic attack, smoking, peripheral arterial disease, and other cardiovascular diseases (except myocardial infarction and atrial fibrillation), resulting in a maximum score of 9 points [5]. To assess the effect of plaque enhancement, detected by perfluorobutane microbubble-CEUS, on the prediction of future recurrent stroke, an additional 1 point was given when the plaque enhancement was detected, to make a combined maximum ESRS plus CEUS score of 10 points.

Follow-Up

After carotid CEUS examination, 149 patients were followed up by checking their medical records or by phone contact with the patients (or their relatives, in cases where the patients had cognitive or language impairments). The starting point of follow-up was the date of onset of the recent stroke, and the end event was stroke recurrence. Stroke recurrence was defined as the presence of a new acute infarct on the side consistent with plaque, as assessed by diffusion magnetic resonance imaging. When magnetic resonance imaging data were not available for patients with suspected recurrent stroke, the duration and characteristics of the new neurological deficit symptoms were used to determine the occurrence of the outcome event. Of 149 patients with carotid atherosclerotic plaques, 19 (12.8%) were lost to follow-up without any available records. Therefore, 130 patients (87.2%) were included in the analysis.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp.). The count data are expressed as frequency (in percentage) and further analyzed using the chi-square test. The Kolmogorov–Smirnov test was used to determine whether the data were normally distributed. Variables that expressed a normal distribution are described as mean ± standard deviation and were tested using Student's *t*-test. The remaining variables that expressed a skewed distribution are described as medians (interquartile), and the Mann–Whitney U test was performed. The Kaplan–Meier method was used to calculate recurrence-free survival in patients with and without plaque enhancement. Statistical differences were determined using the log-rank test. Univariable and multivariable Cox proportional hazards regression analyses were conducted to analyze the risk factors for future stroke recurrence. We assessed the reclassification of risk categories by ESRS plus CEUS compared with ESRS alone, using the net reclassification improvement (NRI) formula [23]. A P-value < 0.05 was considered to represent statistical significance.

RESULTS

Patient Characteristics

The mean age ± standard deviation of the 130 patients (Table 1) included in the final analysis was 62 ± 10 years, and 102 patients (78.5%) were male. Among the 130 patients, 66 (50.8%) had left-sided lesions and the rest had right-sided lesions. There were 108 cases (83.1%) of mild stenosis, 15 cases (11.5%) of moderate stenosis, and 7 cases (5.4%) of severe stenosis. Twenty-five patients (19.2%) experienced recurrent stroke during a follow-up period of 19 ± 4 months, with no deaths recorded. The baseline characteristics of patients with and without recurrent stroke are shown in Table 1.

Plaque Characteristics

The clinical and US characteristics of the study cohort, stratified according to IPN grade, are presented in Table 2. We observed a higher incidence of irregular plaque (P = 0.002) and soft plaque (P = 0.001) in patients with plaque enhancement (IPN grade 2) than in patients without plaque enhancement (IPN grade 1). The length, thickness, remodeling index of the plaque, and plaque burden in patients with plaque enhancement (IPN grade 2) were greater than those in patients without plaque enhancement (IPN grade 1) (all P < 0.05).

Analysis of Risk Factors for Recurrence of Stroke

Table 3 shows the results of univariable and multivariable

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Table 1. Patient Characteristics

	Recurrence Group (n = 25)	Non-Recurrence Group (n = 105)
Age, yr	60 ± 10	62 ± 10
Sex		
Male	21 (84.0)	81 (77.1)
Hypertension	8 (32.0)	52 (49.5)
BMI > upper normal limit	4 (16.0)	14 (13.3)
Diabetes mellitus	12 (48.0)	52 (49.5)
Smoking history	15 (60.0)	58 (55.2)
Coronary artery disease	4 (16.0)	16 (15.2)
Systolic BP, mm Hg	132 ± 20	156 ± 140
Diastolic BP, mm Hg	83 ± 14	84 ± 13
Medication		
Aspirin	4 (16.0)	15 (14.3)
Beta-blocker	2 (8.0)	7 (6.7)
Calcium channel blockers	3 (12.0)	14 (13.3)
Statin	2 (8.0)	8 (7.6)
Oral hypoglycemic agent/ Insulin	6 (24.0)	25 (23.8)
WBC > upper normal limit	1 (4.0)	6 (5.7)
hsCRP > upper normal limit	15 (60.0)	63 (60.0)
TG, mmol/L	1.5 ± 1.0	1.7 ± 1.5
TC, mmol/L	3.7 ± 0.9	4.0 ± 1.5
LDL-C, mmol/L	2.3 ± 0.7	2.4 ± 0.9
HDL-C, mmol/L	1.0 ± 0.3	1.1 ± 0.6
Glucose, mmol/L	5.7 ± 1.1	6.2 ± 2.3
Plaque location		
Left CA	15 (60.0)	51 (48.6)
Right CA	10 (40.0)	54 (51.4)
Plaque position		
Anterior	14 (56.0)	43 (41.0)
Lateral	3 (12.0)	21 (20.0)
Posterior	8 (32.0)	41 (39.0)
Plaque size, mm		
Length	11.5 (8.3–18.8)	14.1 (11.3–21.2)
Thickness	2.8 (2.0–3.2)	2.8 (2.4–3.4)
Plaque echogenicity		
Soft	18 (72.0)	84 (80.0)
Mixed	5 (20.0)	16 (15.2)
Hard	2 (8.0)	5 (4.8)
Calcified	0	0
Carotid lumen diameter, mm	8.2 (6.9–9.8)	8.2 (7.4–9.6)
Plaque ulcer	1 (4.0)	2 (1.9)
Irregular plaque	1 (4.0)	4 (3.8)
Severe carotid stenosis (≥ 70%)	2 (8.0)	5 (4.8)
Plaque enhancement	2 (100.0)	4 (80.0)
Mild and moderate stenosis (< 70%)	23 (92.0)	100 (95.2)
Plaque enhancement	20 (87.0)	47 (47.0)
Plaque burden	0.54 (0.44–0.68)	0.48 (0.34–0.62)
Remodeling index	1.5 (1.4–1.7)	1.0 (0.9–1.1)
Eccentricity index	0.7 (0.6–0.8)	0.7 (0.7–0.8)
Plaque growth mode		
Concentric	1 (4.0)	3 (2.9)
Eccentric	24 (96.0)	102 (97.1)
Plaque enhancement	22 (88.0)	51 (48.6)

Data are presented as number of patients (%), mean ± standard deviation, or median (interquatile range). BMI = body mass index, BP = blood pressure, C = cholesterol, CA = carotid artery, hsCRP = high-sensitivity C-reactive protein, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TG = triglyceride, TC = total cholesterol, WBC = white blood cell count

analyses of the predictors of stroke recurrence. Patients with plaque enhancement (grade 2) on CEUS had an increased risk of stroke recurrence events (22/73, 30.1%) compared with patients without plaque enhancement (grade 1) on CEUS (3/57, 5.3%), with an unadjusted hazard ratio of 55.157 (95% confidence interval [CI]: 30.047–135.523; $P < 0.001$) (Fig. 3). In the final multivariable Cox proportional hazards model analysis, the presence of carotid plaque enhancement was found to be a significant and independent predictor of recurrent stroke, with an adjusted hazard ratio of 38.264 (95% CI: 14.975–97.767; $P < 0.001$).

ESRS vs. ESRS Plus CEUS in Risk Categorization for Stroke Recurrence

Patients at high-risk according to the ESRS had an increased risk of stroke recurrence (22/97, 22.7%), compared to patients with an ESRS low-risk (3/33, 9.1%), with an hazard ratio of 1.706 (95% CI: 0.810–9.014, log-rank $P = 0.048$) (Fig. 4A). When the IPN grade was added to the ESRS, the hazard ratio for stroke recurrence in the high-risk group compared to the low-risk group (2.188; 95% CI, 0.025–3.388]) was greater than that in the ESRS alone group (1.706; 95% CI, 0.810–9.014) (Fig. 4B).

We explored up- or down-reclassification for each risk category by adding the IPN grade. Among patients with low-risk ESRS (n = 33), two (6.1%) were appropriately reclassified to the high-risk category. Applying the NRI formula that considers both those correctly reclassified and those incorrectly reclassified, a net of 32.0% of the recurrence group was reclassified upward appropriately by the addition

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Table 2. Factors Associated with Plaque Enhancement

	Patients without Enhancement (n = 57)	Patients with Enhancement (n = 73)	*P*
Clinical characteristics			
Age, yr	60 ± 10	63 ± 10	0.585
Sex			
Male	40 (70.2)	62 (84.9)	0.090
Hypertension	25 (43.9)	35 (47.9)	0.764
BMI > upper normal limit	9 (15.8)	9 (12.3)	0.523
Diabetes mellitus	28 (49.1)	36 (49.3)	0.800
Smoking history	28 (49.1)	45 (61.6)	0.219
Coronary artery disease	9 (15.8)	11 (15.1)	0.850
Systolic BP, mm Hg	140 ± 25	160 ± 167	0.286
Diastolic BP, mm Hg	83 ± 13	84 ± 13	0.794
Medication use			
Aspirin	8 (14.0)	11 (15.1)	0.869
Beta-blocker	4 (7.0)	5 (6.8)	0.970
Calcium channel blockers	7 (12.3)	10 (13.7)	0.812
Statin	4 (7.0)	6 (8.2)	0.799
Oral hypoglycemic agent/Insulin	12 (21.1)	19 (26.0)	0.509
Laboratory test results			
WBC > upper normal limit	4 (7.0)	3 (4.1)	0.440
hsCRP > upper normal limit	37 (64.9)	41 (56.2)	0.219
TG, mmol/L	1.9 ± 1.9	1.5 ± 0.9	0.116
TC, mmol/L	4.2 ± 1.8	3.7 ± 1.1	0.317
LDL-C, mmol/L	2.5 ± 0.8	2.3 ± 0.9	0.889
HDL-C, mmol/L	1.0 ± 0.2	1.1 ± 0.7	0.241
Glucose, mmol/L	5.9 ± 1.8	6.2 ± 2.4	0.134
Ultrasonography features			
Plaque location			
Left CA	28 (49.1)	38 (50.1)	0.612
Right CA	29 (56.9)	35 (47.9)	0.612
Plaque position			
Anterior	26 (45.6)	30 (41.1)	0.606
Lateral	6 (10.5)	18 (24.7)	0.048
Posterior	25 (43.9)	24 (32.9)	0.200
Plaque size, mm			
Length	12.8 (10.4–15.4)	15.0 (11.4–22.9)	0.039
Thickness	2.5 (2.3–2.8)	3.2 (2.7–4.0)	0.003
Plaque echogenicity			
Soft	33 (57.9)	59 (80.8)	0.001
Mixed	10 (17.5)	11 (15.1)	0.003
Hard	14 (24.6)	3 (4.1)	0.001
Calcified	0	0	-
Carotid lumen diameter, mm	8.0 (7.0–9.3)	8.3 (7.6–9.7)	0.460
Plaque ulcer	1 (1.8)	2 (2.7)	0.710
Irregular plaque	1 (1.8)	4 (5.5)	0.002
Severe carotid stenosis (≥ 70%)	6 (10.5)	1 (1.4)	0.002
Plaque burden	0.40 (0.28–0.52)	0.54 (0.39–0.69)	< 0.001
Remodeling index	1.0 (1.0–1.2)	1.1 (0.9–1.4)	< 0.001
Eccentricity index	0.7 (0.6–0.7)	0.8 (0.7–0.8)	0.237
Plaque growth mode			
Concentric	2 (3.5)	2 (2.7)	0.801
Eccentric	55 (96.5)	71 (97.3)	0.801
Stroke recurrence	3 (5.3)	22 (30.1)	< 0.001

Data are presented as number of patients (%), mean ± standard deviation, or median (interquatile range). BMI = body mass index, BP = blood pressure, C = cholesterol, CA = carotid artery, hsCRP = high-sensitivity C-reactive protein, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TG = triglyceride, TC = total cholesterol, WBC = white blood cell count

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Table 3. Risk Factors for Ischemic Stroke Recurrence

Variable	Univariable Analysis			Multivariable Analysis		
	Unadjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Age, yr†	0.980	0.944–1.019	0.311	-	-	-
Sex (male vs. female)*	0.662	0.227–1.929	0.450	-	-	-
Hypertension (yes vs. no)*	1.510	0.220–2.182	0.116	-	-	-
BMI > upper normal limit (yes vs. no)*	1.172	0.402–3.415	0.771	-	-	-
Diabetes mellitus (yes vs. no)*	1.074	0.483–2.391	0.861	-	-	-
Smoking history (yes vs. no)*	1.168	0.525–2.601	0.704	-	-	-
Coronary artery disease (yes vs. no)*	1.077	0.370–3.139	0.892	-	-	-
WBC > upper normal limit (yes vs. no)*	0.761	0.103–5.624	0.789	-	-	-
hsCRP > upper normal limit (yes vs. no)*	0.990	0.445–2.203	0.980	-	-	-
TG, mmol/L†	0.843	0.511–1.288	0.429	-	-	-
TC, mmol/L†	0.856	0.610–1.202	0.370	-	-	-
LDL-C, mmol/L†	0.849	0.530–1.358	0.493	-	-	-
HDL-C, mmol/L†	0.814	0.290–2.286	0.695	-	-	-
Plaque location (left CA vs. Right CA)*	1.425	0.640–3.173	0.386	-	-	-
Plaque position (anterior vs. non-anterior)*	1.725	0.782–3.804	0.177	-	-	-
Length of plaque, mm†	0.974	0.921–1.031	0.364	-	-	-
Thickness of plaque, mm†	0.990	0.681–1.439	0.958	-	-	-
Plaque echogenicity (soft vs. mixed, hard and calcified)*	1.219	0.509–2.920	0.656	-	-	-
Carotid lumen diameter, mm†	0.992	0.817–1.204	0.933	-	-	-
Plaque ulcer (yes vs. no)*	23.021	0.000–20596799	0.654	-	-	-
Irregular plaque (yes vs. no)*	1.142	0.154–8.442	0.897	-	-	-
Severe carotid stenosis (≥ 70%) (yes vs. no)*	1.508	0.444–2.156	0.358	-	-	-
Plaque burden, degree†	1.302	0.184–9.235	0.792	-	-	-
Remodeling index, degree†	50.675	20.795–123.487	< 0.001	1.401	0.110–2.452	0.164
Eccentricity index, degree†	0.777	0.105–5.725	0.804	-	-	-
Plaque growth mode (concentric vs. eccentric)*	2.009	0.600–6.729	0.258	-	-	-
Plaque enhancement (yes vs. no)*	55.157	30.047–135.523	< 0.001	38.264	14.975–97.767	< 0.001

*For categorical variables with categories in parentheses, the former was compared with the latter (the reference) to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) with the Cox regression analysis, †For continuous variables, an increase by 1 considered when calculating HRs and 95% CIs. BMI = body mass index, C = cholesterol, CA = carotid artery, hsCRP = high-sensitivity C-reactive protein, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TG = triglyceride, TC = total cholesterol, WBC = white blood cell count

of the IPN grade to the ESRS (Table 4, Fig. 5).

DISCUSSION

Patients with a recent ischemic stroke are at risk of a second ischemic stroke. Our findings revealed that carotid plaque enhancement on perfluorobutane microbubbles-CEUS was a significant and independent predictor of stroke recurrence. Furthermore, the detection of carotid plaque enhancement improved the risk stratification capability of the ESRS in predicting stroke recurrence. These findings have important clinical implications in that noninvasive carotid CEUS may be used for the risk stratification of patients with recent stroke.

Previous studies have revealed that the IPN of carotid plaques, as characterized by SonoVue CEUS, is associated with ischemic stroke recurrence in patients with carotid atherosclerosis [15,16]. In a recent meta-analysis, Huang et al. [24] analyzed 20 studies that used CEUS to identify neovascularization within plaques, four of which used quantitative methods, and found that both qualitative and quantitative methods had good diagnostic accuracy, but qualitative assessments had higher diagnostic performance than quantitative ones. Therefore, we performed qualitative rather than quantitative analysis of the CEUS examination results. This dichotomy is simple and repeatable [24]. In our study, carotid plaque enhancement was a significant and independent predictor of stroke recurrence in patients with a

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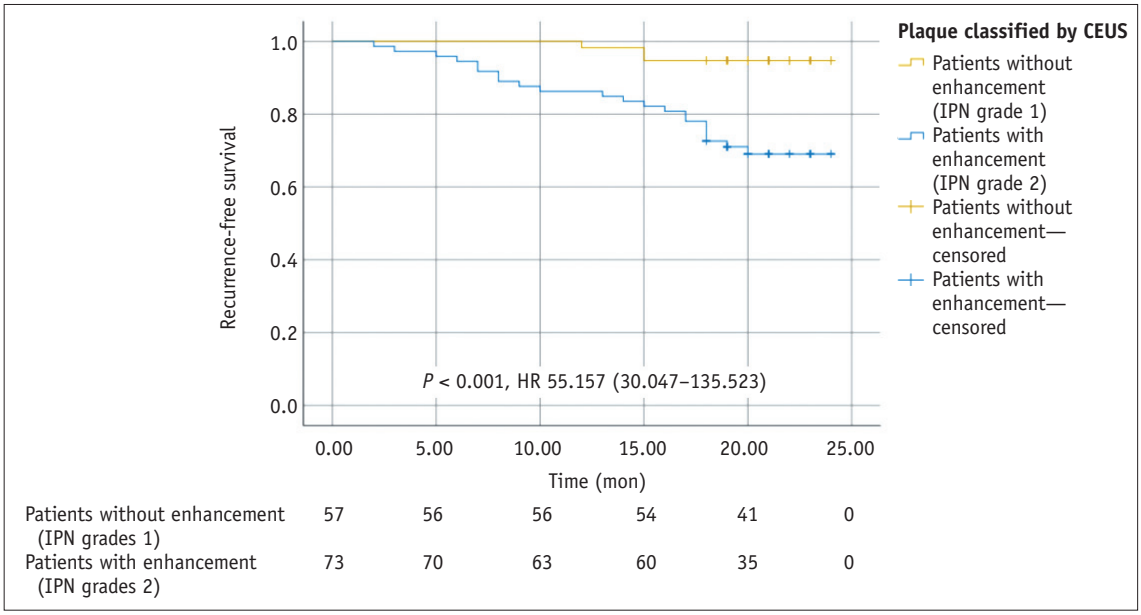

Fig. 3. Kaplan–Meier recurrence-free survival curves according to the contrast-enhanced ultrasonography (CEUS) findings. Recurrence-free survival in patients with plaque enhancement is significantly worse than that in those without plaque enhancement. HR = hazard ratio, IPN = intraplaque neovascularization

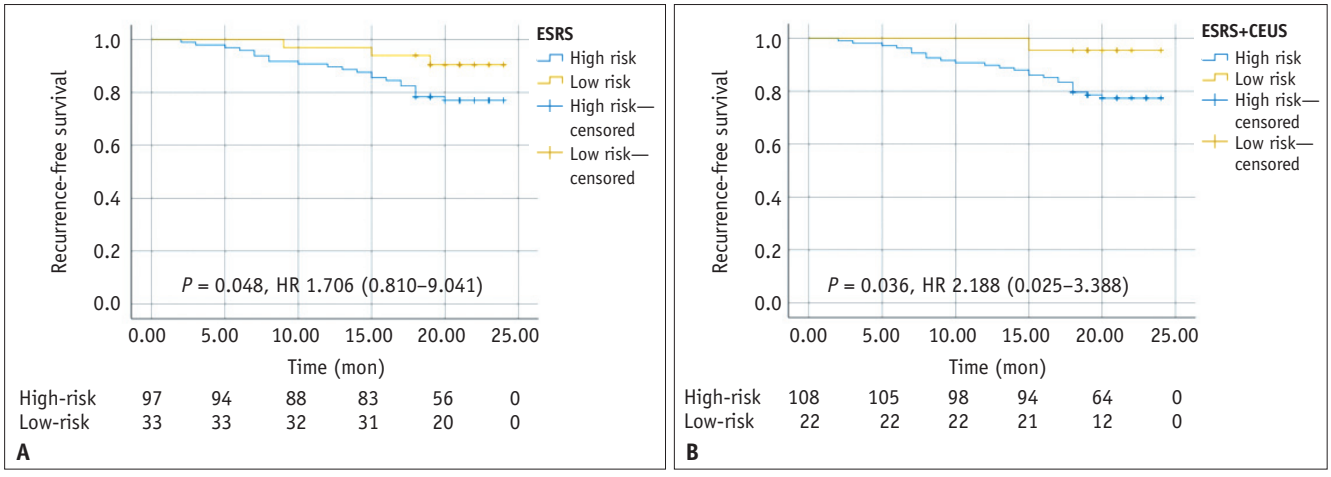

Fig. 4. Kaplan–Meier recurrence-free survival curves according to risk groups by Essen Stroke Risk Score (ESRS) and ESRS + contrast-enhanced ultrasonography (CEUS). **A.** Recurrence-free survival in the high-risk group is significantly worse than that in the low-risk group classified by the ESRS alone. **B.** Recurrence-free survival in the high-risk group is significantly worse than that in the low-risk group classified by CEUS plus ESRS. Note that when the presence of plaque enhancement was added to the ESRS, the hazard ratio (HR) for stroke recurrence was higher in high-risk groups than that of ESRS alone.

recent ischemic stroke, which is consistent with the results of previous studies [15,16] .

Our study extended these previous observations by demonstrating the added value of IPN grade on CEUS to ESRS for the prediction of recurrent stroke. According to the ESRS, age, hypertension, diabetes mellitus, myocardial infarction, prior stroke or transient ischemic attack, active smoking, peripheral arterial disease, and other cardiovascular diseases were independent predictors of first and recurrent stroke [25]. Our study confirmed its ability to discriminate between high-risk and low-risk groups for developing recurrent stroke by showing that cumulative recurrence-free survival in high-risk patients according to the ESRS was significantly worse than that in low-risk patients. However, the ESRS does not include information on atherosclerotic plaques detected using noninvasive imaging technologies. To predict stroke recurrence, we believe that the IPN may provide additional information about carotid artery plaque,

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Table 4. Net Reclassification Improvement for Stroke Recurrence Prediction with the Addition of the IPN Grades to the ESRS

Classification According to the ESRS	Classification According to the ESRS + IPN Grades		
	Low-Risk	High-Risk	Total
Low-risk (< 3)			
Nonevents	21	9	30
Events	1	2	3
NRI, %			
Nonevents	-	40.0	-
Events	-	33.3	-
Overall	-	73.3	-
High-risk (≥ 3)			
Nonevents	0	75	75
Events	0	22	22
NRI, %			
Nonevents	-	-100	-
Events	-	100	-
Overall	-	0	-
Total			
Nonevents	21	84	105
Events	1	24	25
NRI, %			
Nonevents	-	-60.0	-
Events	-	92.0	-
Overall	-	32.0	-

ESRS = Essen Stroke Risk Score, IPN = intraplaque neovascularization, NRI = net reclassification improvement

which may help stratify the risk of atherosclerosis-related stroke recurrence. Both hazard ratio and area under the curve improved when the IPN grade was added to the ESRS. Our study showed that two of three patients at low risk of developing recurrent stroke according to the ESRS were upward-reclassified by the addition of the IPN grade. This finding suggests that IPN grade can help in the early identification of high-risk patients, despite the absence of traditional risk factors. To date, no study has combined CEUS and ESRS to stratify the risk of future stroke recurrence in the carotid plaque population.

Our results also indicated that the remodeling index of the recurrence group was higher than that of the non-recurrent group. In atherosclerotic vessel walls, an increase in plaque content is associated with compensatory enlargement of the vessel wall, thereby maintaining the effective area in the lumen. Positive remodeling (remodeling index ≥ 1.0) is associated with abundant macrophages and proliferating necrotic centers, and suggests the presence of symptomatic plaques [25]. Previous studies have shown that the assessment of plaque inflammation can effectively predict early stroke recurrence [26]. IPN aggravates the inflammatory response in the plaque, which further promotes IPN formation [27]. Our findings are consistent with the hypothesis that the IPN and plaque inflammation contribute

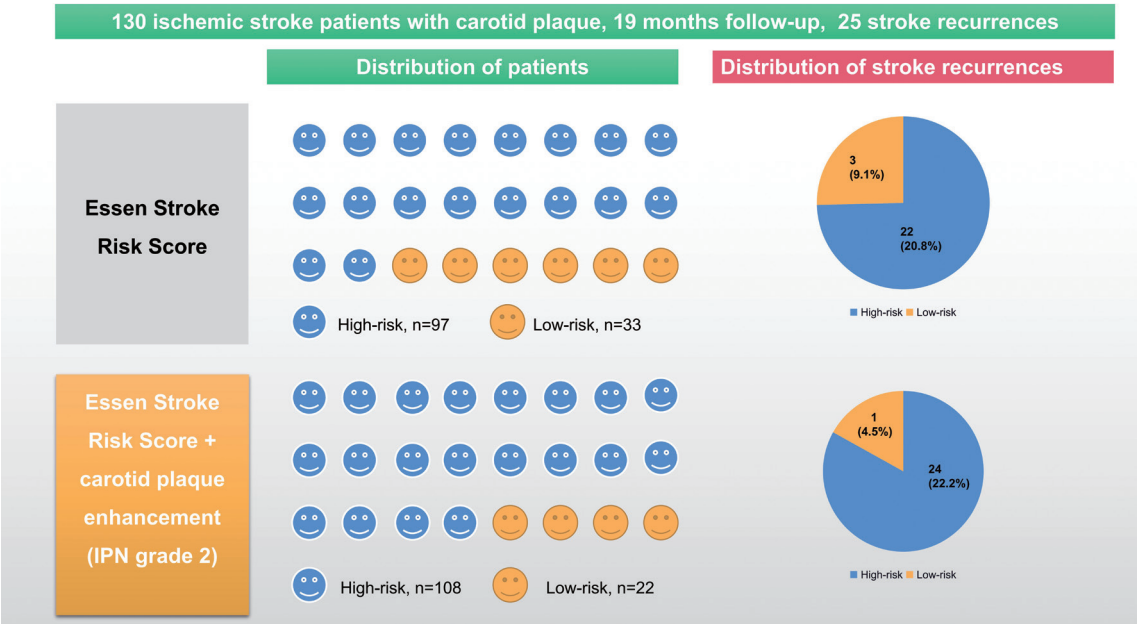

Fig. 5. Added value of carotid plaque enhancement (intraplaque neovascularization [IPN] grade 2) on Essen Stroke Risk Score for prediction of stroke recurrences. Over 19 months of follow-up, among 130 patients with carotid plaque, stroke recurrences occurred in 25 patients. Among patients with a low risk on the Essen Stroke Risk Score (n = 33), 2 (6.1%) were appropriately reclassified into the high-risk category.

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to plaque progression.

ESRS is one of the predictive tools to judge the risk of stroke recurrence based on the ischemic stroke population, which can predict the occurrence of stroke and complex cardiovascular events [28]. The CHADS2 is a scale used to assess stroke risk in patients with atrial fibrillation [29], but not all of our study subjects had atrial fibrillation. ABCD2 has been widely validated as a risk assessment tool for stroke recurrence within 2–90 days of the onset of transient ischemic attack [30]; however, our follow-up was 19 ± 4 months. The Stroke Prognostic Instrument (SPI)-II was used to assess long-term recurrence risk in patients with ischemic stroke [31].

Our study had some limitations. First, the follow-up period was relatively short. Future studies with a longer follow-up period are needed to study the contribution of carotid plaque enhancement determined by perfluorobutane microbubble-CEUS to SPI-II. Second, the sample size was small. Third, we assessed only the thickest plaques, possibly ignoring the small vulnerable plaques that could contribute to stroke. Finally, CEUS relies heavily on image planes. Therefore, neovascularization may have been overlooked in some plaques. Large multicenter studies with longer follow-up periods are needed to validate the current findings.

In conclusion, carotid plaque enhancement is a significant and independent predictor of stroke recurrence in patients with recent ischemic stroke. Furthermore, the addition of carotid IPN improved the risk stratification capability of the ESRS, suggesting that noninvasive carotid CEUS may be used for risk stratification in patients with recent stroke.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Zhe Huang, You-Bin Deng. Data curation: Zhe Huang, Xue-Qing Cheng, You-Bin Deng. Formal analysis: all authors. Investigation: all authors. Methodology: all authors. Project administration: all authors. Resources: all authors. Software: Zhe Huang. Supervision: Ya-Ni Liu, Xiao-Jun Bi, You-Bin Deng. Validation: all authors. Visualization: all authors. Writing—original draft: all authors. Writing—review & editing: all authors.

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