Predictive Value of Carotid Plaque Contrast-Enhanced Ultrasound Score and Homocysteine in Senile Metabolic Syndrome Complicated by Cerebral Infarction

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ABSTRACT

Objective: To investigate the predictive value of the carotid plaque contrast-enhanced ultrasound (CEUS) score and blood homocysteine (HCY) in senile metabolic syndrome (MetS) complicated by cerebral infarction.

Study Design: Observational study.

Place and Duration of the Study: Department of Ultrasound Imaging, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, China, from July 2020 to December 2021.

Methodology: A total of 118 senile MetS patients complicated by cerebral infarction were selected as Group A, and 103 senile MetS patients without cerebral infarction were selected as Group B. Both groups were compared in terms of cardiovascular risk factors and ultrasonic examination of carotid plaques. The independent risk factors for cerebral infarction among senile MetS patients were analysed using logistic regression. An ROC curve was used to assess the predictive value of statistically significant risk factors in senile MetS complicated by cerebral infarction.

Results: Significant differences were observed in smoking, abdominal circumference, blood pressure, HCY, fasting blood glucose, high-density and low-density lipoprotein cholesterol, triacylglycerol, carotid plaque thickness, CEUS score, lumen stenosis, and ulcer plaque between the two groups. Logistic regression analysis showed that the plaque CEUS score and HCY were independent risk factors for senile MetS complicated by cerebral infarction. The areas under the ROC curve for the CEUS score and HCY were 0.795 and 0.812, respectively, and was 0.858 for the combined diagnosis of both. When the CEUS score was ≥2 and HCY was ≥16.45 mmol/l, the sensitivity and specificity of predicted senile MetS complicated by cerebral infarction were 83.1% and 74.8%, respectively.

Conclusion: The carotid plaque CEUS score and blood HCY exhibit a substantial predictive capacity for cerebral infarction in elderly MetS patients. The combined diagnostic efficacy of the two is superior.

Key Words: *Contrast-enhanced ultrasound, Homocysteine, Elderly, Metabolic syndrome, Cerebral infarction, Carotid plaque*.

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INTRODUCTION

Metabolic syndrome (MetS) is a pathological state of abnormal aggregation of a variety of risk factors associated with metabolism, including hypertension, abdominal obesity, dyslipidemia, and diabetes.[1] They are often closely linked and affect each other in a vicious cycle, which significantly increases the risk of cardiovascular disease and cancer.[2]

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In recent years, the prevalence of MetS among the elderly population in China has been steadily rising, owing to the enhanced living standards and the progressively evident demographic ageing. MetS is an important risk factor for cerebral infarction, and the incidence of cerebral infarction is higher in senile MetS patients.[3] The higher morbidity and disability rates pose a great threat to their health and quality of life. Therefore, it is very important for the early identification and prevention of cerebral infarction risk in elderly patients with metabolic syndrome.

Homocysteine (HCY) is an intermediate in cysteine-methionine metabolism. Elevated blood HCY concentrations can directly damage the vascular endothelium, induce oxidative stress and inflammatory responses, and promote atherosclerotic plaques and thrombosis.[4] Consequently, an increased concentration of HCY is a risk factor for the development of cardiovascular and cerebrovascular disorders.[5] Lee *et al.* reported a noteworthy association between hyper-HCY and MetS, with elevated HCY levels exerting a causative influence on the progression of MetS.[6] Srećković B *et al.* found that the level of HCY was not only related to the components of MetS but also aggravated with age, and MetS-related HCY increased the risk of cardiovascular disease.[7] In contrast, others found that although blood HCY levels in patients with MetS were significantly higher than those in non-MetS groups, there was no significant correlation between this high level of HCY and new cardiovascular events.[8,9] The reasons for the different results of these studies may be related to the design of the study, the inclusion of age and race of the population, and the different diagnostic criteria of MetS. Currently, blood Hcy and cardiovascular events in Chinese elderly patients with MetS are rarely studied.

Cardiovascular risk factors play an important role in the formation and progression of atherosclerosis. It has been found that microemboli formed by the rupture of unstable carotid plaques and haemorrhage are important in the pathogenesis of cerebral infarction.[10] Lyu *et al.* confirmed that pathological neovascularisation in plaques was closely related to plaque instability through histological examination of plaques in patients with carotid endarterectomy.[11] Contrast-enhanced ultrasound is the main method to detect neovascularisation in plaques.[12] The Chinese Contrast-enhanced Ultrasound Clinical Application Guidelines recommend the use of the carotid plaque CEUS score to semi-quantitatively evaluate the degree of plaque neovascularisation.[13] According to the degree of plaque enhancement, the score of neovascularisation was 0-3. The higher the plaque score, the more neovascularisation, and the more unstable, the plaque. However, for carotid atherosclerotic plaques in elderly MetS patients with multiple risk factors, no cut-off value of the CEUS score had been established to predict the risk of cerebral infarction.

Based on a comprehensive analysis of clinical and ultrasonic data from elderly patients with MetS, this study aimed to evaluate the predictive value of the carotid plaque CEUS score combined with blood HCY in senile MetS complicated by cerebral infarction.

METHODOLOGY

The study was approved by the Ethics Committee of the Affiliated Hospital of Traditional Chinese Medicine of Southwest Medical University. Informed consent forms were signed by all participants. From July 2020 to December 2021, 118 patients with MetS complicated by cerebral infarction and 103 patients with MetS without cerebral infarction were divided into Group A and Group B. The inclusion criteria was MetS patients aged ≥60 years, and carotid plaque thickness was >1.5 mm without recent intake of vitamins B12, B6, and folic acid. All patients underwent routine carotid ultrasound and CEUS. The exclusion criteria was peripheral vascular occlusive disease, cardiogenic cerebral infarction, heart failure, pulmonary oedema and other serious diseases, ultrasound contrast agent allergy, and plaque strong echo components ≥ 20%, which may affect the angiographic results.

Following admission, the clinical data of the patients were gathered and documented, as shown in Table I.

Diagnosis of MetS was made if three or more conditions were present: Abdominal obesity (central obesity), circumference of the waist of a man ≥90 cm or a woman ≥85 cm, hypertension (blood pressure ≥ 130/85 mmHg and/or people with diagnosed and treated hypertension). Hyperglycaemia was defined as fasting plasma glucose (FPG) ≥6 mmol/L or 2h after glucose load ≥7.8 mmol/L and/or people with diabetes who have been diagnosed and treated. Hypercholesterolemia was defined as triacylglycerol (TG) ≥1.70 mmol/L at fasting, and high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L at fasting.[1]

The diagnostic criteria for cerebral infarction were implemented based on the Chinese guidelines for acute ischemic stroke.[14] The study included individuals who were newly diagnosed with cerebral infarction.

For the ultrasonic examination method; the authors used the L9-3 MHz linear-array probe (Resona8; Mindray, Shenzhen, China) to perform standard carotid ultrasound on all patients. According to the Mannheim consensus,[15] carotid intima-media thickening >1.5 mm was defined as plaque. The maximum plaque thickness was measured on the short axis section, and the maximum length was measured on the long axis. The ulcer plaque was sunken on the surface of the plaque, with a clear boundary, length and depth ≥2 mm. According to the North American Radiological Association consensus on carotid artery stenosis diagnosis,[16] the degree of stenosis was divided into three groups: mild (<50% or normal), moderate (50-69%), severe (70-99%) or occlusion. In Group A, the thickest plaque on the ipsilateral side of the cerebral infarction was analysed, and in Group B, the thickest plaque was analysed.

CEUS used SonoVue contrast medium (Bracco, Italy), diluted with 0.9% sodium chloride injection 5 ml, shaken and set aside. The probe was placed at the plaque, and the mechanical index was reduced to a low level (0.1). The lumen was immediately rinsed with 5.0 ml of normal saline after 2.4 ml of contrast medium SonoVue was injected through the cubital vein. The dynamic images were stored in double display mode, and the plaque enhancement signs and their degrees were recorded. The reference standard of the carotid plaque neovascularisation score was as follows: 0 points = no enhancement within the plaque; 1 point = spot enhancement on the adventitia and/or shoulder of the plaque; 2 points = the plaque has 1-2 linear or multiple punctate enhancements; and 3 points = plaque linear enhancement over a large area, penetrating or mostly penetrating plaques, or blood flow signs.[13] Two doctors with more than five years of CEUS experience analysed the images, and if there were differences, the final decision was discussed. Two doctors had a good repetition of the CEUS score of carotid plaques: their ICC was greater than 0.90 out of 20 random plaques.

SPSS 25.0 and MedCalc 20.64 were used to analyse the data. Variables were assessed for normality using normality tests and

Kolmogorov–Smirnov tests. Normally distributed data were presented as x ± s, and an independent sample t-test was conducted; nonnormally distributed data were expressed as the median and quartile range, and the Mann–Whitney U test was used to compare each group. The categorical variables were expressed in terms of sample size and frequency (%), and the appropriate Chi-square test was used for comparison between the groups. Taking cerebral infarction as a dependent variable and a variable with a difference in univariate analysis as a covariable, multivariate logistic regression was used to analyse the independent risk factors for cerebral infarction in senile MetS. The sensitivity and specificity of the CEUS score and HCY in predicting senile MetS complicated by cerebral infarction were determined in light of the ROC curve. The value $p < 0.05$ was considered to be statistically significant.

RESULTS

Comparing the two groups of general data, smoking, abdominal girth, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, HCY, TG, low-density lipoprotein cholesterol (LDL-C), and cholesterol (TC) levels were higher in Group A, while HDL-C levels were lower ($p < 0.05$, Table I).

Looking at the routine ultrasound and CEUS for carotid plaque, Group A had thicker carotid plaques and more ulcer plaques than Group B ($p < 0.05$). The plaque CEUS scores of the two groups were significantly different ($p < 0.01$), with group A scoring mostly 2 and 3 and Group B scoring 0 and 1. In both groups, the degree of carotid stenosis differed significantly ($p < 0.05$). Compared to Group B, Group A had a higher proportion of moderate and severe stenosis and a lower proportion of mild stenosis (Figure 1 and Table II).

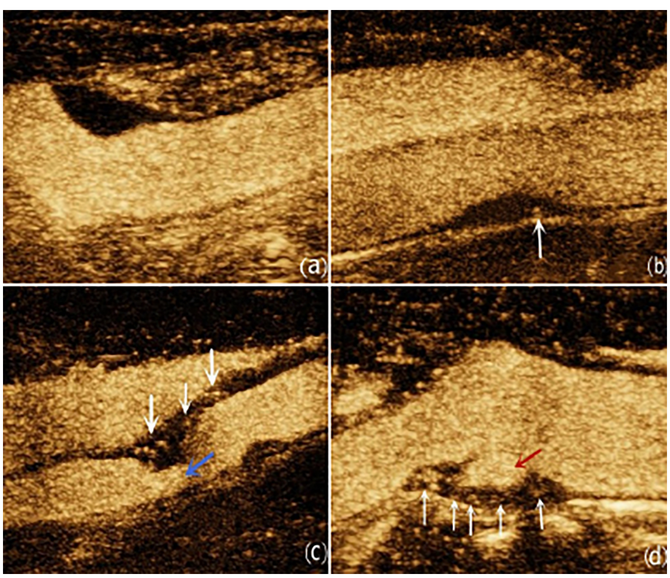

Figure 1: Contrast-enhanced ultrasonography and scoring of carotid plaque. (a) No enhancement seen within the plaque, 0 points. (b) Spot enhancement seen in the plaque (white arrow), 1 point. (c) The plaque has 1-2 linear or multiple punctate enhancements (white arrows), 2 points. The blue arrow indicates moderate stenosis of the lumen caused by plaque. (d) Plaque linear enhancement over a large area, penetrating or mostly penetrating plaques (white arrows), 3 points. The red arrow indicates ulceration on the plaque surface.

The multivariate logistic regression analysis was conducted, considering cerebral infarction as the dependent variable and including smoking, abdominal girth, SBP, DBP, FPG, HCY, TG, LDL-C, TC, HDL-C, plaque CEUS score, plaque ulceration, and lumen stenosis as covariates. The results indicated that blood HCY and plaque CEUS scores were independent risk factors for cerebral infarction among elderly MetS patients ($p<0.001$, $p=0.001$, respectively), while HDL-C was a protective factor ($p=0.021$, Table III).

The ROC curve analysis indicated that the AUC (the area under the ROC curve) of the plaque CEUS score for predicting cerebral infarction in elderly MetS patients was 0.795 (95% CI: 0.735-0.856, $p < 0.001$), the optimal threshold was 2, and the corresponding sensitivity and specificity were 85.6% and 68.0%, respectively. The AUC of blood HCY for predicting cerebral infarction in elderly MetS patients was 0.812 (95% CI: 0.755-0.870, $p < 0.001$), the optimal threshold was 16.45 mmol/L, and the corresponding sensitivity and specificity were 80.5% and 73.8%, respectively. The AUC of the above two combined diagnoses was 0.858 (95% CI: 0.809-0.906, $p < 0.001$), and the sensitivity and specificity of predicting cerebral infarction in elderly MetS patients were 83.1% and 74.8%, respectively. The combination of the two had a higher diagnostic efficiency (Figure 2).

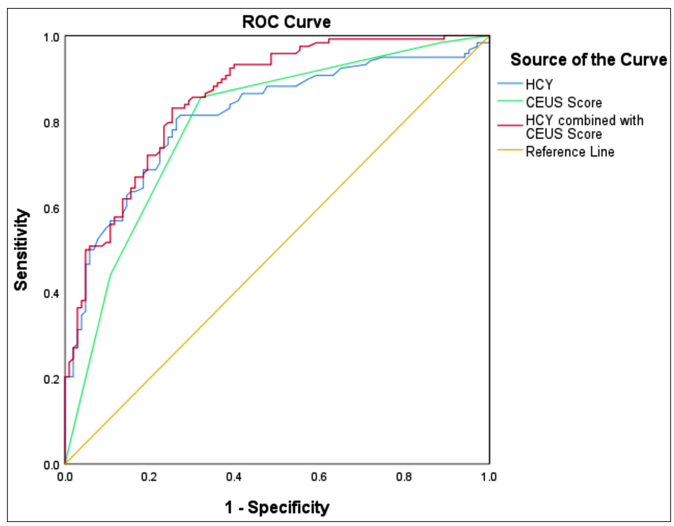

Figure 2: ROC curve of CEUS score, HCY levels alone and their combination in predicting senile MetS complicated by cerebral infarction.

DISCUSSION

In recent years, the prevalence of MetS in developing nations has continued to rise, especially among elderly individuals, thus posing a serious public health problem.[17] Senile patients with low self-awareness of the disease, poor prevention and treatment, and even the use of hypotension, hypoglycaemia, statins and other drugs often do not adjust the metabolic indicators to the ideal level. In this study, the prevalence of 5 metabolic component abnormalities in the two groups was high, and statistical significance was not found. However, in Group A, the abnormalities in abdominal circumference, SBP, DBP, FPG, TG, TC, HDL-C, and LDL-C were more significant than in Group B, and all of the differences were statistically significant.

Table I: Comparing the general data of the two groups.

Project	Group A (n = 118)	Group B (n = 103)	p-value
Gender [n (%)]			0.780 *
Male	70 (59.3%)	63 (61.2%)	
Female	48 (40.7%)	40 (38.8%)	
Age (year)	70.05±6.04	68.71±5.56	0.089 #
Smoking [n (%)]	44 (37.3%)	25 (24.3%)	0.037 *
Drink alcohol [n (%)]	27 (22.9%)	22 (21.4%)	0.786 *
Abdominal obesity [n (%)]	91 (77.1%)	75 (72.8%)	0.460 *
Hypertension [n (%)]	103 (87.3%)	84 (81.6%)	0.239 *
Diabetes [n (%)]	96 (81.4%)	76 (73.8%)	0.177 *
Dyslipidaemia [n (%)]	96 (81.4%)	81 (78.6%)	0.614 *
Abdominal girth	97.85±11.00	94.49±9.41	0.016 #
SBP	154.78±22.37	138.56±21.65	<0.01 #
DBP	91.71±10.77	84.08±11.49	<0.01 #
FPG	7.75 (5.60-9.60)	5.50 (4.90-7.80)	<0.01 △
TG	2.29 (1.55-4.84)	1.58 (1.39-2.46)	<0.01 △
HDL-C	1.06 (0.67-1.25)	1.22 (0.87-1.53)	<0.01 △
LDL-C	3.79 (2.77-5.79)	2.92 (2.47-4.33)	0.001 △
TC	5.42 (4.61-7.15)	5.12 (4.54-5.86)	0.024 △
HCY	21.90 (18.00-29.00)	14.70 (13.20-17.60)	<0.01 △
Statins [n (%)]	36 (30.5%)	43 (41.7%)	0.082 *
Antihypertensive drugs [n (%)]	48 (40.7%)	53 (51.5%)	0.109 *
Antidiabetic drugs [n (%)]	43 (36.4%)	48 (46.6%)	0.126 *

**Chi-square test; # Independent samples t-test; △ Mann–Whitney U test.*

Table II: Comparison of carotid plaque routine ultrasound and CEUS scores between the two groups.

	Group A (n = 118)	Group B (n = 103)	p-value
Thickness (mm)	3.95±1.06	3.41±0.96	<0.01 #
Length (mm)	15.89±3.54	15.42±3.93	0.353 #
CEUS Score [n (%)]			<0.01 △
0	2 (1.7%)	12 (11.7%)	
1	15 (12.7%)	58 (56.3%)	
2	49 (41.5%)	22 (21.3%)	
3	52 (44.1%)	11 (10.7%)	
Lumen stenosis [n (%)]			0.031 △
Mild	66 (55.9%)	70 (68.0%)	
Moderate	30 (25.5%)	25 (24.2%)	
Severe	22 (18.6%)	8 (7.8%)	
Ulceration [n (%)]	19 (16.1%)	6 (5.8%)	0.016 *

*# Independent samples t-test; △ Mann–Whitney U test; * Chi-square test.*

Table III: Analysis of multivariate logistic regression in senile MetS complicated by cerebral infarction.

Risk factor	B	p-value	OR	95% CI	
				Lower limit	Upper limit
HDL-C	-1.433	0.021	0.238	0.071	0.804
HCY	0.273	<0.001	1.314	1.184	1.458
Plaque CEUS score	1.036	0.001	2.817	1.550	5.122

It was suggested that cerebral infarction in patients with MetS was not related to the disease itself but was related to the abnormal degree of metabolic components. However, in the results of multivariate regression analysis, except for HDL-C as a protective factor for cerebral infarction, none of the other metabolic syndrome components were independently associated with the increased risk of cerebral infarction. This was because adverse events in patients with MetS may be due to complex interactions between abnormal metabolic components and not just based on expectations of purely additive effects.[18]

The occurrence of atherosclerosis in patients with MS is closely related to high homocysteine levels that aggravates with age.[7] In this study, the concentration of blood HCY were elevated in both groups, with Group A exhibiting a significantly higher level of HCY compared to Group B, which was consistent with the study of Azarpazhooh *et al*.[19] The increased level of HCY serve as an autonomous risk factor in the development of atherosclerotic plaques and ischemic stroke.[5] The toxic effects of peroxide and oxygen-free radicals produced during the metabolism of hyperhomocysteinemia on endothelial cells can reduce the activity of nitric oxide synthase, induce the expression of thromboxane A2 and oxidative stress, and then lead to the formation and development of atherosclerosis. At the same time, the aggregation of homocysteine lipoprotein and microorganisms blocks the lumen in the process of atherosclerotic plaque formation, leading to plaque hypoxia and thrombosis.[4,20] The research conducted by Song *et al.* demonstrated a positive association between blood HCY levels and microvessel density in carotid atherosclerotic plaques, thereby influencing plaque stability.[21] The findings of the multivariate regression analysis conducted in this study indicated that blood HCY level served as an independent risk factor for cerebral infarction among elderly patients with MetS, suggesting that the higher the level of HCY in elderly patients with MetS, the more unstable the plaque and the higher the possibility of cerebral infarction. ROC curve analysis showed that a blood HCY concentration ≥16.45 mmol/L was the best critical value of serum HCY for predicting cerebral infarction in senile patients with MetS, and its AUC was 0.812.

Vascular damage caused by major cardiovascular risk factors is mainly manifested in the occurrence and development of atherosclerotic and atherosclerotic plaques.[22] Metabolic syndrome is a combination of these risk factors. Bos *et al.* found that neovascularisation in carotid atherosclerotic plaques was strongly associated with accidental stroke.[23] Neovascularisation in the plaque has serious developmental abnormalities and structural defects, such as lack of basement membrane, brittleness, easy leakage, induction of macrophage aggregation and aggravation of the inflammatory reaction in the plaque. Plaque haemorrhage and rupture induced by fragile neovascularisation are important factors leading to plaque instability.[24] Contrast-enhanced ultrasound is the primary method for detecting neovascularisation in plaques. In this study, neovascularisation was evaluated according to the CEUS score recommended by Chinese Contrast-enhanced Ultrasound Clinical Application guidelines.[13] The results showed that the CEUS scores of plaques in Group A were mainly 2 and 3, while those in Group B were mainly 0 and 1. The CEUS score in Group A was significantly higher than that in Group B. Additionally, Group A demonstrated higher levels of plaque thickness, ulcer plaque detection rate, and proportion of moderate and severe stenosis in comparison to Group B. The multivariate regression analysis demonstrated that the CEUS score served as an independent risk factor for cerebral infarction among elderly patients with MetS, suggesting that the higher the CEUS score of plaques, the richer the neovascularisation in plaques, the more unstable plaques, and elderly MetS patients were at greater risk of cerebral infarction. The ROC curve analysis showed that a carotid plaque CEUS score ≥2 was the best critical value for predicting cerebral infarction in elderly patients with MetS, and its AUC was 0.795.

In this study, the AUC, sensitivity and specificity of the carotid plaque CEUS score combined with blood HCY in predicting cerebral infarction in elderly patients with MetS were 0.858, 83.1%, and 74.8%, respectively. A combined diagnosis can more accurately identify elderly MetS patients with the risk of cerebral infarction than a single index, which can enhance clinical vigilance toward these patients and carry out active and effective intervention, promptly and effectively, to prevent the occurrence of any adverse events.

There are certain limitations to the generalisation of the results of this study. First, the thickest plaque selected was not necessarily the plaque responsible for disease progression. However, the study conducted by Spence *et al.* demonstrated that the stability of the largest plaque and cerebral infarction were significantly correlated.[25] Secondly, this study was conducted in elderly MetS patients with a high-positive rate of carotid plaque; therefore, the results of this study may not be suitable for carotid ultrasound screening in the general population.

CONCLUSION

The carotid plaque CEUS score and blood HCY exhibit a substantial predictive capacity for cerebral infarction in elderly MetS patients, with the combined diagnostic efficacy of the two being superior.

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ETHICAL APPROVAL:
This study was approved before its start by the Ethics Committee of the Affiliated Hospital of Traditional Chinese Medicine of Southwest Medical University, Sichuan, China, (Decision No. YJKY2020049 dated: 7/14/2020).

PATIENTS' CONSENT:
Informed consents were obtained from the patients to publish the data.

COMPETING INTEREST:
The authors declared no competing interest.

AUTHORS' CONTRIBUTION:
ZZ: Conception, design, data collection, data analysis, and manuscript drafting.
ZX: Establishing the theoretical framework, critical review, and proofreading.
CC: Data collection, manuscript preparation, and editing.
SY: Data collection and analysis.
ML: Review, critical revision, and supervision of the manuscript, accountable for all aspects of the work.
All authors approved the final version of the manuscript to be published.

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