Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Carotid Siphon Calcification Predicts the Symptomatic Progression in Branch Artery Disease With Intracranial Artery Stenosis—Brief Report

Duanlu Hou, Xiaoli Yang, Yuanyuan Wang, Shengwen Huang, Yuping Tang, Danhong Wu

BACKGROUND: Arterial calcification in the aortic arch, carotid bifurcation, or siphon on computed tomography was associated with cardiovascular disease. The association between arterial calcification prevalence and progression of branch atheromatous disease (BAD) in intracranial artery atherosclerosis was little investigated.

METHODS: This study included 310 patients with ischemic stroke from one stroke center. Patients were divided into BAD (110) and non-BAD groups (200). Baseline characteristics, lipids, and arterial calcification were measured. The primary outcome was the prevalence of arterial calcification in BAD progression, and the secondary outcome was the prevalence of calcification in arterial stenosis. The association or correlation among calcification prevalence, lipid markers, and BAD progression was analyzed using logistic regression, receiver operating characteristic curve, and linear regression.

RESULTS: Our study found that carotid siphon calcification on computed angiography was more prevalent (P=0.01) in patients with BAD and also more prevalent (P<0.001) in intracranial artery stenosis, and its computed tomography values could independently predict the symptomatic progression (P=0.01). Furthermore, a strong linear correlation between oxidized lipid and calcification density was found (beta=−0.73, P=0.0048) in patients with BAD, a subtype (B-type) of intracranial arterial atherosclerotic disease.

CONCLUSIONS: We found that carotid siphon calcification was associated with BAD and its computed tomography values could predict the symptomatic progression in patients with intracranial arterial atherosclerotic disease and BAD, indicating the important role of carotid calcification in B-type intracranial arterial atherosclerotic disease.

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Key Words: angiography ■ atherosclerosis ■ carotid artery ■ low-density lipoprotein ■ stroke

Arterial calcification especially in coronary and carotid arteries has important biomechanical consequences on circulatory function in the heart and brain. Vulnerable atherosclerotic plaques with calcification always rupture and lead to cardiovascular or cerebrovascular diseases.[1] Age, current smoking, hypercholesterolemia, and history of hypertension or diabetes are independently related to calcification in coronary, aortic arch, and carotid arteries.[2] Arterial calcification in patients with stroke increases the risk of atherosclerotic stroke independent of age, sex, and atrial fibrillation.[3,4] Intracranial calcification is associated with the early progression or recurrence of stroke, coronary events, vascular deaths,[5] and hemorrhagic transformation in patients with noncardioembolic stroke.[6] Vertebrobasilar calcification in patients with ischemic stroke is a valid predictor for stroke recurrence and acute vascular disease.[7] Coronary artery calcification in patients with atrial fibrillation has

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Nonstandard Abbreviations and Acronyms

BAD	branch atheromatous disease
hsCRP	high-sensitivity C-reactive protein
LOX1	lectin-like oxidized low-density lipoprotein receptor-1
NIHSS	National Institutes of Health Stroke Scale
sLOX1	soluble LOX1

Highlights

- We reported that carotid siphon calcification can predict the symptomatic progression of branch artery disease.
- We reported the strong correlation between oxidized LDL (low-density lipoprotein) and carotid calcification in patients with B-type intracranial artery atherosclerotic disease.

increased the independent risk of stroke,[3] but carotid calcification is not associated with incident stroke.[8]

Characteristics of arterial calcification were associated with the stability of atherosclerosis. For example, the intimal type of calcification is the most prevalent subtype of intracranial atherosclerosis.[4] And the medial-type of calcification, with internal soft plaque, is highly predictive of intraplaque hemorrhage.[9] Macrocalcification is associated with inflammatory factors as well as proteoglycan 4 which is typical for the stability of atherosclerosis[10]; however, microcalcification is associated with unstable plaque and intraplaque hemorrhage in the carotid artery.[11–13]

The Agatston calcium score can evaluate the coronary calcification[14] and predict incident carotid plaque vulnerability and intracranial artery stenosis.[15] The prediction and discrimination of computed tomography (CT) are better than that of ultrasound.[16,17]

A recent study showed that LOX1 (lectin-like oxidized low-density lipoprotein receptor-1) ligand containing apo AI was associated with coronary calcification in Japanese males,[18] indicating the underlying mechanism between LOX1 and calcification. Another study[19] and our unpublished results showed the associations between LOX1, oxidized lipoprotein, and the progression of coronary atherosclerosis and branch atheromatous disease (BAD); this inspired us to investigate the association between calcification, oxidized lipids, and progression of BAD, the disease now regarded as a vulnerable plaque-related disease affecting the small penetrating artery.[20]

We aim to investigate the associations between different characteristics (CT density, size, thickness, and scores), different types of calcification in the carotid siphon, aortic arch or carotid bifurcation and the BAD progression, and also the relationship between BAD and intracranial arterial stenosis and then the underlying mechanism of LOX1-related lipids between calcification and BAD.

MATERIALS AND METHODS

Data Available Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient Enrollment and Study Design

Consecutive patients with mild ischemic stroke were screened and selected from the Stroke Center of Shanghai Fifth People's Hospital (The Fifth People's Hospital of Shanghai, Fudan University) between April 1, 2017, and April 1, 2020. Patient selection was according to the inclusion criteria and exclusion criteria (see the Supplemental Material).

Figure shows a flow diagram of the selected patients and the final number of the patients who have met the required sample size (see the Supplemental Material). Written informed consent was obtained from all patients or their families. This study was approved by the Ethical Review Board of the Shanghai Fifth People's Hospital before patient enrollment.

Baseline Data Collection

Baseline data were collected from medical records including age, sex, National Institutes of Health Stroke Scale (NIHSS) scores, systolic blood pressure, and history of hypertension, diabetes, atrial fibrillation, dyslipidemia, cigarette smoking, and alcohol consumption. All blood samples were collected in vacuum tubes, stored at 4 °C, and tested by clinical laboratory technicians in a certified laboratory within 2 hours after collection. Homocysteine, HDL (high-density lipoprotein), LDL (low-density lipoprotein), total cholesterol, total triglyceride, and hsCRP (high-sensitivity C-reactive protein) concentrations were measured on the second day of admission.

Measurement of Lipoprotein Markers

Fasting venous plasma samples were obtained at 6:00 on the second day after admission and before discharge (10–14 days after stroke onset) to measure oxidized (ox) LDL and sLOX1 (soluble LOX1) levels (see the Supplemental Material).

Clinical Assessments

Each enrolled patient underwent a head 3-T magnetic resonance imaging, magnetic resonance angiography, or 64-slice CT (Siemens, Forchheim, Germany) and CT angiography (Canon, Tokyo, Japan) within 48 hours after stroke onset.

Two neurologists evaluated the magnetic resonance imaging images and clarified the diagnosis of BAD according to lesion shape and vascular supply.[21,22] The criteria were (1) the admission within 48 hours after onset, (2) an ischemic lesion, which was confirmed by the diffusion-weighted imaging on admission, localized in the cerebral lenticulostriate arterial region or in the pontine penetrating arterial region, involving three or more levels in the horizontal position, (3)

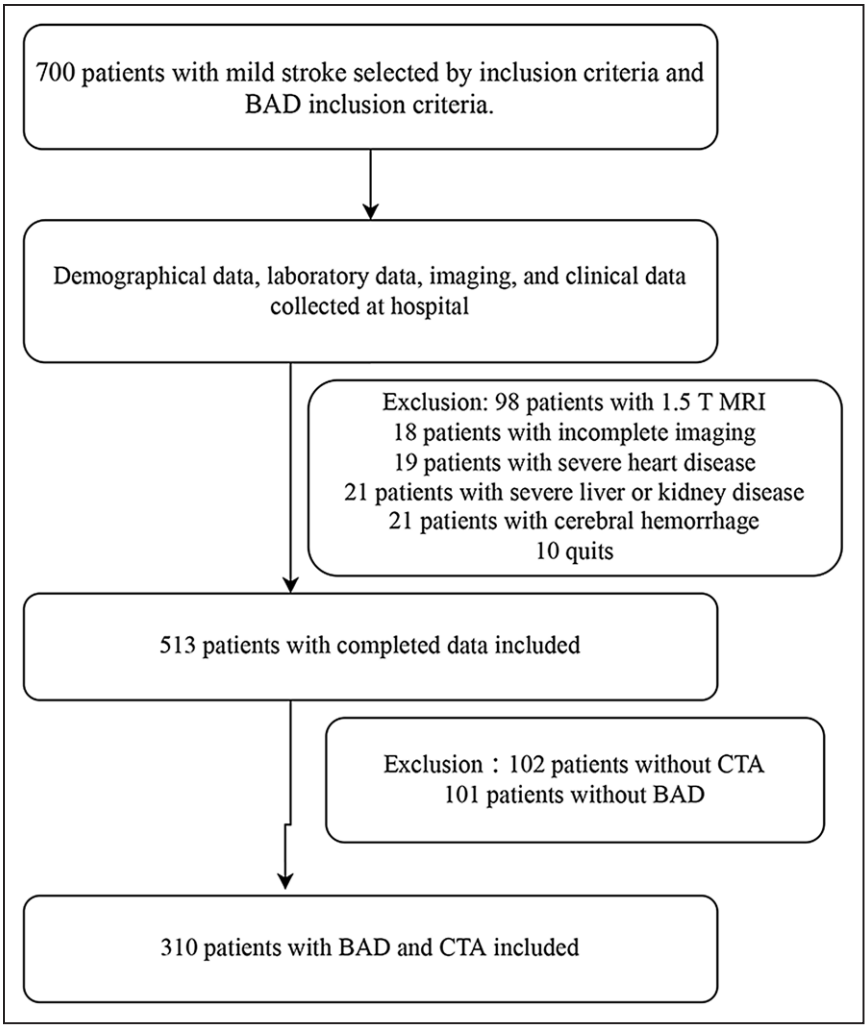

Figure. Flowchart of participant selection.
BAD indicates branch atheromatous disease; CTA, computed tomography angiography; and MRI, magnetic resonance imaging.

no evidence of cardioembolism or other causes, according to the Japan Branch Atheromatous Disease Registry criteria[23] and Chinese criteria (shown in the Supplemental Material). The progression of BAD or progressive BAD (pBAD) was defined as an exacerbation of the symptoms within 48 to 72 hours of the patient's admission, which can be detected on objective examination and changes of NIHSS scores (ΔNIHSS>4)[24] made by the neurologists. pBAD mainly refers to the remarkable symptom progression in BAD and belongs to the category of BAD.

Artery calcification of aortic arch, bilateral carotid siphon, and bilateral carotid bifurcation, and the CT density, thickness (plaque length), and size (length×width×height) of the carotid siphon calcification on CT angiography were measured by two independent neurologists (calcium appeared as high density on CT angiography). Intracranial artery stenosis in magnetic resonance angiography was measured by the radiologist and neurologist by calculating the ratio of long diameters of the plaque and the artery.

Infarct Size Evaluation

Infarct volume was measured using diffusion-weighted imaging and apparent diffusion coefficients were used to distinguish between acute and nonacute signals (see the Supplemental Material). The ITK-SNAP software (Version 3.8.0) was used to measure the acute infarct volumes indicated by diffusion-weighted imaging 16 (http://www.itksnap.org/pmwiki/pmwiki.php). The images were independently analyzed by 2 blinded stroke neurologists (D.H. and X.Y.), and the inter-rater reliability was 0.86. The average calculated from the volumes analyzed by 2 neurologists was used for further analysis.

Statistical Analysis

SPSS 26.0 (IBM Corp, Armonk, New York), and GraphPad Prism 8 (GraphPad Software, Inc, La Jolla, CA) was used to perform statistical analyses (see the Supplemental Material). Patients were dichotomized according to the presence of pBAD and the best cutoff value of calcification CT values (Hounsfield unit) and intracranial artery stenosis of different groups were tested using the Mann-Whitney *U* test.

RESULTS

Characteristics

Over 3 years, 310 patients with BAD and with NIHSS score of ≤5 were included from 700 enrolled patients of the cohort in the analysis. These patients were divided into 2 groups according to the existence of

Table. Comparisons Between Patients With BAD With or Without Symptomatic Progression

	With progression (n=110)	Without progression (n=200)	*P* value
Demographic data			
Age, y*	67 (60–76)	66 (59–73)	0.67
Male	60 (55%)	120 (60%)	0.30
Hypertension	90 (82%)	170 (85%)	0.46
Diabetes	50 (45%)	92 (46%)	0.20
Smoking	35 (32%)	94 (47%)	0.09
Drinking	24 (22%)	48 (24%)	0.65
Onset-to-MRI time, min*	74 (37–94)	69 (47–97)	0.90
Infarction size, mL*	2.74 (1.58–8.33)	1.78 (1.0–4.1)	0.01†
Admission NIHSS*	4 (2–5)	4 (2–5)	0.80
Laboratory data*			
TC, mmol/L	4.0 (3.3–4.6)	4.3 (3.7–5.0)	0.02†
TG, mmol/L	1.29 (0.9–1.9)	1.39 (0.9–1.9)	0.44
HDL, mmol/L	1.0 (0.9–1.2)	1.0 (0.8–1.3)	0.81
LDL, mmol/L	2.73 (1.9–3.2)	2.79 (2.2–3.5)	0.13
HCY, μmol/L	13.6 (11–17)	12.4 (11–18)	<0.001†
hsCRP, mg/L	0.75 (0–2.5)	1.05 (0.4–3.3)	0.01†
sLOX1, mmol/L	2.60 (2.2–3.1)	2.59 (1.7–3.1)	0.26
oxLDL, mmol/L	247.5 (214–311)	230.7 (179–295)	0.09
Mean arterial stenosis ratio, %	61 (50–70)	35 (24–46)	0.04†
Intracranial stenosis severity			
None (0)	44 (40%)	92 (46%)	0.51
Mild (<50%)	10 (9%)	54 (27%)	0.02†
Moderate (≧50%–70%)	37 (34%)	36 (18%)	0.04†
Severe (≧70%)	19 (17%)	18 (9%)	0.03†
Intracranial artery stenosis ≧50%	100 (91%)	74 (37%)	<0.001†
Middle cerebral artery	63 (63%)	62 (84%)	0.003†
Basilar artery	37 (37%)	6 (8%)	<0.001†
Other	0 (0%)	6 (8%)	0.005†
Artery calcification			
Aortic arch	33 (30%)	70 (35%)	0.46
Carotid bifurcation	24 (22%)	36 (18%)	0.49
Carotid siphon	41 (37%)	40 (20%)	0.01†
Calcium size, mm^3*	4.09 (1.99–6.82)	5.57 (1.81–8.23)	0.65
CT density, Hu*	524 (397–617)	629 (499–759)	0.04†
CT×size, $Hu \cdot mm^3$*	1595 (754–4268)	3531 (832–6416)	0.24
Intimal calcium	36 (88%)	4 (10%)	<0.001†
Thickness, mm*	1.61 (1.05–1.95)	1.75 (1.24–2.29)	0.51

Unless specified, values are numbers of patients (%). BAD indicates branch atheromatous disease; CT, computed tomography; HCY, homocysteine; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HU, Hounsfield unit; LOX, soluble lectin-like oxidized low-density lipoprotein receptor; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; oxLDL, oxidized low-density lipoprotein; TC, total cholesterol; and TG, total triglyceride.

*Median (interquartile range).

†Statistically significant.

pBAD (Table), patients with pBAD have no differences in age, sex, history of hypertension, diabetes, smoking, drinking, and the onset-to-magnetic resonance imaging time. Patients with pBAD have larger infarction volumes (P=0.01), higher intracranial artery stenosis rate (P<0.001), and higher mean stenosis ratio (P=0.04) compared with those without pBAD. But the admission NIHSS scores in both groups showed no difference (P=0.80). In subgroup analysis of stenosis rate, patients with progression have lower middle cerebral

artery stenosis rate (P=0.003), higher basilar artery stenosis (P<0.001), higher severe intracranial artery stenosis (P=0.03), and higher carotid siphon calcification rate (P=0.01) than patients without progression.

Carotid Siphon Calcification Density Is Associated With Symptomatic Progression in Patients With BAD

In the comparison of calcification characteristics of carotid siphon between the 2 groups in the Table, patients with symptomatic progression have lower CT values (Hounsfield unit; P=0.04), and higher intimal calcium rate (P<0.001). But the size and thickness of the calcification showed no difference.

Intracranial Stenosis Is Associated With Symptomatic Progression in Patients With BAD

In comparison between patients with intracranial stenosis and those without intracranial stenosis (Table S1), patients with progression had a higher prevalence of stenosis (P<0.001). However, patients with progression had a lower prevalence of middle cerebral artery stenosis (P=0.003) and a higher prevalence of basilar artery stenosis (P<0.001) than patients without progression.

Carotid Siphon Calcification Is Associated With Intracranial Stenosis in Patients With BAD

In comparison of calcification between patients with stenosis and without stenosis (Table S1), calcification in carotid siphon (P<0.001), and carotid bifurcation (P=0.002) was more prevalent in patients with stenosis. The size (P=0.03) and CT (P=0.02) value of calcification in carotid siphon were higher in patients with stenosis.

Carotid Siphon Calcification Density Independently Predicts the Symptomatic Progression in pBAD

A multivariate regression analysis of the calcification-related biomarkers for the occurrence of pBAD was performed. In the analysis with pBAD occurrence as the outcome variable, we found that the lower CT value of the calcium in the carotid siphon was the independent risk factor for the occurrence of pBAD, but age, homocysteine, total cholesterol, LOX1, oxLDL (oxidized LDL), hsCRP, infarct size, and admission NIHSS scores were not. Table S2 shows that carotid siphon calcification density was independently associated with pBAD occurrence (odds ratio, 1.23 [95% CI, 1.05–1.44]; P=0.01) after adjusting for confounders.

Receiver Operating Characteristic Curve for Discriminating pBAD From Non-pBAD

Receiver operating characteristic analysis identified the best cutoff value, 557 Hounsfield unit for carotid siphon calcification density to discriminate pBAD from non-pBAD. Receiver operating characteristic analysis of the calcification density showed that the area under the curve was 0.85, with 95% CI of 0.75–0.90 (P<0.0001; Youden index=0.49; sensitivity, 73%; specificity, 76%; Figure S1).

Correlation Between oxLDL and Calcification Density in Carotid Siphon

The present study found a negative linear correlation between oxLDL levels and calcification density of carotid siphon (Figure S2), the equation of the regression analysis was CT=−0.728×oxLDL+779.4, the beta values were −0.73 (with 95% CI of −1.2 to −0.2), and P=0.0048. This correlation showed a good association between oxLDL and calcification formation. Furthermore, taking the results together, higher oxLDL levels seemed to be associated with lower CT density in the carotid siphon and then lower CT density was associated with pBAD.

DISCUSSION

Our study found that carotid siphon calcification density on CT angiography could independently predict the symptomatic progression and also was related to intracranial artery stenosis in patients with BAD, a subtype (B-type) of intracranial arterial atherosclerotic disease.

Nowadays, studies have reported that the pathology of BAD was atherosclerosis and atherosclerosis progression,[25] but calcification in BAD was little investigated. The severity of large artery stenosis was associated with BAD.[26] Also, the vulnerable plaque with intraplaque hemorrhage and calcification were independently associated with middle cerebral artery stenosis.[27] Plaques in coronary and carotid arteries were more vulnerable than those in renal and iliac arteries.[28] The intimal and medial types of carotid calcification had same prognostic values on cerebrovascular disease in the territory of the internal carotid artery.[29] The Rotterdam Study reported that stroke prevalence was related to carotid calcification but not to aortic or coronary calcification.[30] The Multi-Ethnic Study of Atherosclerosis study found the insignificance of the aortic calcification in predicting cardiovascular disease with zero coronary calcium.[31,32] Carotid bifurcation calcium size was associated with carotid stenosis and carotid plaque vulnerability.[33,34] The existence of carotid siphon calcium was associated with the incidence and severity of lacunar stroke[35] but not a reliable marker of carotid stenosis.[36]

Our study found that the aortic arch and carotid bifurcation calcification were not related to the BAD progression. However, carotid siphon calcification prevalence was higher in patients with BAD and its CT values were lower in patients with BAD progression. These findings suggested the important role of carotid siphon calcification in predicting symptomatic progression in patients with BAD. In addition, our study found that intracranial arterial stenosis was more common in patients with pBAD, and carotid calcification especially carotid siphon calcification was more common in patients with arterial stenosis. This indicated the role of carotid siphon calcification in B-type intracranial arterial atherosclerotic disease. B-type intracranial arterial atherosclerotic disease was the most prevalent subtype of large artery atherosclerosis, and its pathophysiology was vulnerable plaque affecting the penetrating arteries.[37,38] The present study demonstrated that the calcification in carotid siphon had a prognostic value on BAD progression, and the calcification severity was a good predictor for disease progression in patients with atherosclerotic plaque.

A study on heart valve calcification in 1997[39] found that ethanol preincubation for lipid extraction could prevent calcium formation, which firstly indicated the lipid theory in calcification formation.[40] Nowadays the roles of HDL, LDL, and VLDL (very low-density lipoprotein) in vascular calcification are documented.[41,42] In addition, serum homocysteine was independently associated with intracranial arterial calcification[43] and homocysteine was also related to the incidence of BAD,[44] which indicated the association between homocysteine, calcification, and BAD. Our study found a strong correlation between lipid oxidation (especially after adjusting homocysteine levels) in atherosclerosis and calcium density with the beta value of −0.73 and demonstrated the role of oxidized lipid in calcification formation: the higher the oxLDL levels, the lower the calcium density. This indicated the solid plaque or calcification plaque with high CT values (Hounsfield unit) was associated with lower lipid oxidation (ie, oxLDL) in atherosclerosis. oxLDL is a significant factor that enhances coagulation in atherosclerosis, contributes to plaque progression,[45] and inhibits the removal of apoptotic cells.[46] Furthermore, our results found no association between solid calcification and calcium size or intimal thickness, showing the less important role of size in calcification.

The study focused on the association between calcification and atherosclerotic disease. (1) The calcification density was associated with BAD progression, a disease affecting the cerebral small artery. (2) Large arterial stenosis was more prevalent in patients with BAD progression. Furthermore, (3) calcification was correlated with lipid oxidation (oxLDL). So, (4) calcification might be related to vulnerable plaque and also to BAD with arterial stenosis. All conclusions to some extent demonstrated the vital role of carotid calcification in large arterial, atherosclerotic disease (stenosis) representing small vessel damage (or BAD). Future studies need large cohorts with follow-up data on stroke recurrence and functional outcomes with a large sample size and studies on the association between lipid oxidation markers and calcification formation.

There are some limitations to the present study. First, the present cohort study was conducted in a single stroke center and recruited only the Chinese population with small sample size. A larger number of population samples, including cohorts of different ethnic, geographic, and age groups, need to be conducted in the future. And only patients with mild stroke were included. Because the subject of our study is the progression in mild stroke, not macrovascular stroke progression. In mild stroke progression, the vast majority are BAD, and some BAD lesions are fully consistent with the complete penetrating arterial territory (multilevel, along the vessel alignment in magnetic resonance imaging), and some BAD lesions resemble small lacunar infarcts (incomplete infarction). In addition, patients with BAD tend to have NIHSS scores between 0 and 5,[47] which meet the definition of mild stroke. And severe stroke with higher NIHSS scores (>5) tends to be cardioembolic stroke or acute large artery occlusion. Second, we only measured the levels of LOX1 and oxLDL; other oxidative stress lipids, such as L5 and dysfunctional HDL, should be measured in any such future analyses. Nevertheless, LOX1 and oxLDL have been identified as key factors that play a major role in the atherosclerotic process, playing a much greater role than factors such as L5.[48] Third, relevant follow-up data such as NIHSS at 3 months, Barthel index, and cognitive assessments were not included in the study. Given that the study was set in a mild stroke population where NIHSS was determined to be below 5, there was little difference in prognosis. Studies have found good prognostic indicators, such as hyperglycemia, NIHSS score, and infarct volume[49] rather than lipid levels. Therefore, there was no further exploration between the prognosis and lipid-related markers in detail.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

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