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ORIGINAL RESEARCH

Molecular Biomarkers Associated with Early-Onset Symptomatic Intracranial Atherosclerosis

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Purpose: Previous studies have shown a rising incidence of early-onset symptomatic intracranial atherosclerosis (sICAS), which has brought a severe economic burden to social development. This study aimed to evaluate the molecular biomarkers associated with early-onset sICAS and to seek possible intervention strategies for early prevention.

Patients and Methods: We consecutively recruited patients with sICAS and divided them into two groups based on age: early-onset sICAS group as age ≤60 years old and late-onset sICAS group as age >60 years old. We collected and compared the demographic data and laboratory results of each group. A bivariate logistic regression model was applied to evaluate the independent molecular biomarkers of early-onset sICAS.

Results: A total of 1007 subjects with sICAS were enrolled in this study, comprising 519 patients in the early-onset sICAS group and 488 patients in the late-onset sICAS group. Bivariate logistic regression analysis demonstrated an increased level of white blood cell, platelet, albumin globulin ratio, free triiodothyronine, and a decreased level of total bile acid, urea nitrogen, high-density lipoprotein, homocysteine, and fibrinogen in the early-onset sICAS group when compared to the late-onset group.

Conclusion: Our study showed the relevance between early sICAS and circulating levels of different molecular biomarkers. Detection of these related molecular biomarkers may provide a simple way for early sICAS preventions in the future.

Keywords: ischemic stroke, symptomatic intracranial atherosclerosis, molecular biomarkers

Introduction

Intracranial atherosclerosis (ICAS) is the leading cause of ischemic stroke around the world. ICAS could be symptomatic or asymptomatic, and the symptomatic intracranial atherosclerosis (sICAS) refers to the ischemic stroke or transient ischemic attack (TIA) that occurs in the stenosis area of the supplying artery.[1] sICAS is associated with higher rates of severe symptoms, recurrence and rehospitalization.[2–5] A recent study showed that 45%-62% of patients with ischemic stroke had intracranial plaque and stenosis.[6] Generally, age is an independent risk factor for sICAS, and the incidence of sICAS increases in the elderly.[7] However, the occurrence of sICAS has shown a younger trend in recent years. Several studies have shown that risk factors are more complex and patients are more prone to worse prognosis in early-onset sICAS than in late-onset sICAS.[8–10] Early-onset sICAS affects patients' quality of life and ability to work, and thus brings a heavy economic burden to the family and society.

In the past two decades, several researchers have sought to determine biomarkers correlated with sICAS, such as adipokines, interleukin-6, C-reactive protein, and

matrix metalloproteinases.[11] What's more, laboratory indicators including white blood cells, platelets, albumin/globulin ratio, and free triiodothyronine are considered to be independent risk factors for intracranial atherosclerotic diseases.[12–14] The interaction among white blood cells, platelets and endothelial cells is the leading cause of inflammation and atherosclerosis.[15] However, serum albumin can attenuate the oxidative damage of atherosclerotic lesions.[16] High level of preoperative serum-free thyroxine 4 can be used as a sensitive and independent predictor of vascular restenosis in patients with angina pectoris after bare stent implantation.[17] To date, little is known about the prevalence and risk factors of early-onset sICAS. Genetic risk factors like RNF213 p.R4810K variant and RNF213 cg22443212 hypermethylation are reported to be associated with early-onset sICAS.[18,19] However, circulating biomarkers of early-onset ICAS have not been widely explored.

In the present study, we aimed to explore molecular biomarkers associated with early-onset sICAS and seek an intervention tactic for earlier preventions against these factors in the future.

Patients and Methods

Study Population

The flow chart of this study is shown in Figure 1. We consecutively recruited 1007 sICAS patients in Xiangya Hospital of Central South University from August 2012 to May 2019. The diagnosis of ischemic stroke or TIA conforms to the Chinese guidelines for the diagnosis and treatment of acute ischemic stroke in 2018.[20] All patients were classified into two groups based on their age: early-onset group defined as age ≤60 years old, and a late-onset group as age >60 years old. The inclusion criteria were as follows: 1. The age between 18 and 80 years old and the time from symptom-onset to hospital ≤14 days; 2. All the subjects met the criteria for clinical diagnosis of ischemic stroke and transient ischemic attack. We excluded patients with the following clinical characteristics: 1. Patients with cardiogenic stroke or other non-atherosclerotic causes diseases; 2. Imaging examinations indicated no intracranial artery stenosis or extracranial artery stenosis; 3. Other intracranial artery stenosis causes, such as moyamoya disease and vasculitis; 4. The patients have recent diseases, such as infection, tumor and autoimmune rheumatism; 5. Severe liver and kidney

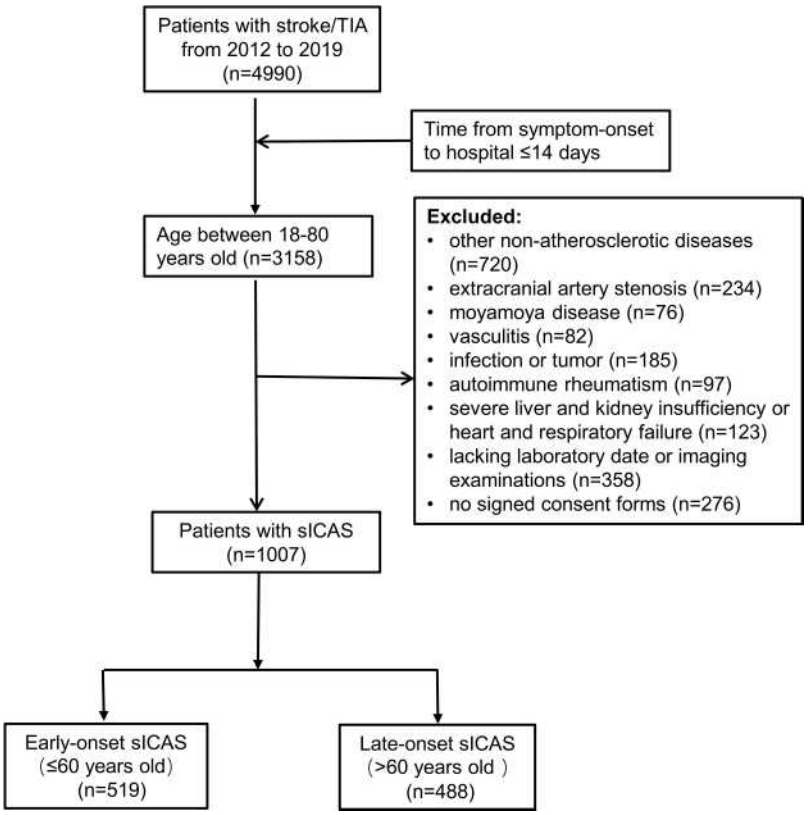

Figure 1 Patient selection flowchart.
Abbreviations: sICAS, symptomatic intracranial atherosclerosis; TIA, transient ischemic attacks.

insufficiency or heart and respiratory failure; 6. Lacking data of laboratory or imaging examinations; 7. None of the signed consent forms. Written informed consent was obtained from each patient included in the study, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Xiangya Hospital of Central South University, China.

Clinical Data Collection

Demographic information included age, sex, stroke history, family history of stroke, vascular risk factors (diabetes mellitus, hypertension, coronary artery disease, dyslipidemia, Table 1), alcohol intake, and cigarette smoking. The patient's peripheral venous blood was drawn in the next morning after admission, and was immediately examined in the Biochemical Laboratory of Xiangya Hospital. We recorded the plasma levels of blood routine, hepatic and renal function, plasma glucose and lipids, blood coagulation, thyroid function, and homocysteine. Most subjects underwent routine MRI examinations in a 3.0T or 1.5T MRI scanner, which composed of three-dimensional time-of-flight magnetic resonance angiography (3D-TOF-MRA), contrast-enhanced magnetic resonance angiography (CE-MRA), T2/T1-weighted magnetic resonance images and fluid attenuated inversion recovery (FLAIR). Computed tomography (CT) and computed tomography angiography (CTA) were used to supplement those who cannot complete or cooperate with MRI. All patients had completed the carotid Doppler angiography. The DSA (gold standard), 3D-TOF-MRA and CTA were performed to distinguish the location and severity of intracranial artery stenosis. The intracranial artery stenosis was determined according to the Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) Study: percent stenosis = $[1-(D_{stenosis}/D_{normal})] \times 100\%$ ($D_{stenosis}$: the diameter of the vessel at the site of stenosis; D_{normal}: the diameter of the normal vessel just distal to the stenosis).[21] The severity of stenosis was divided into four grades: 1. mild stenosis: <50%; 2. moderate stenosis: 50% to 69%; 3. severe stenosis: 70% to 99%; 4. occlusion: no blood flow. The National Institutes of Health Stroke Scale (NIHSS) score was independently evaluated by two experienced clinicians at admission to evaluate the severity of stroke.

Definition of Vascular Indicators

Hypertension was diagnosed when systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or undergoing treatment of antihypertensive medications. Diabetes was diagnosed when fasting blood glucose (FBG) greater than 7mmol/L and/or postprandial glucose (PPG) greater than 11.1 mmol/L or intake of antidiabetic drugs. Dyslipidemia was defined as total cholesterol (TC) ≥5.18 mmol/L or triglyceride (TG) ≥1.70 mmol/L or low-density lipoprotein (LDL) >3.19 mmol/L or high-density lipoprotein HDL <1.04 mmol/L. Stroke history referred to the patients who had incurred a single event of ischemic stroke or transient ischemic attack. Smokers were considered as the subject who had smoked continuously for 1 year with ≥1 cigarette per day. Alcohol consumption was defined as average drinking greater than 2 standards for males or 1 standard for females per day.

Statistical Methods

All statistics were carried out using SPSS 22.0 software (IBM). We denoted categorical variables with absolute values and percentages. Continuous variables were presented as means ± standard deviations (± SD) when conformed to the normal distribution or median (interquartile range (IQR)) when the data were non-normally distributed. To analyze the differences between groups, a chi-square test (χ^2) was performed on categorical covariates, while a Student's *t*-test or Mann-Whitney *U*-test and one-way ANOVA or Kruskal–Wallis test were performed on continuous data. We used the median to classify the continuous variables into lower and higher groups in the bivariate correlation analysis. In the logistic regression analysis, variables with p-values <0.05 in the univariate analyses were included. In the overall statistical analysis, p-values <0.05 were considered statistically significant.

Results

Baseline Characteristics of All Patients

From August 2012 to May 2019, a total of 1007 patients confirmed with sICAS were recruited, 488 patients had early-onset sICAS and 519 patients had late-onset sICAS. The median age was 61 years old (IQR: 52–68 years old), including 645 males (64.1%) and 362 females (35.9%), with a male-to-female ratio of 1.78:1. The median NIHSS score at admission was 4 (IQR: 2–8). Other demographic data and risk factors are shown in Table 1, and the most common cardiovascular risk factor was hypertension (76.4%), followed by smoking (44.1%) and diabetes (33.8%).

Table 1 Baseline Characteristics of sICAS Patients

Variables	Patients (n=1007)
Age (years) (median, IQR)	61 (52–68)
Sex (male, N, %)	645 (64.1%)
Smoking (N, %)	444 (44.1%)
Drinking (N, %)	334 (33.2%)
History	
Stroke History (N, %)	204 (20.3%)
Hypertension (N, %)	769 (76.4%)
DM (N, %)	340 (33.8%)
Dyslipidemia (N, %)	477 (47.4%)
CAD (N, %)	150 (14.9%)
Family History of Stroke (N, %)	51 (5.1%)
Ischemic type	
Stroke (N, %)	954 (94.7%)
TIA (N, %)	53 (5.3%)
WBC ($\times 10^9$/L) (median, IQR)	6.8 (5.6–8.3)
PLT ($\times 10^9$/L) (median, IQR)	204.0 (166.0–248.0)
NLR (median, IQR)	2.7 (1.9–3.8)
AGR (median, IQR)	1.5 (1.3–1.6)
TBIL (μmol/L) (median, IQR)	9.9 (7.5–13.1)
TBA (μmol/L) (median, IQR)	3.5 (2.1–5.9)
BUN (mmol/L) (median, IQR)	4.9 (4.0–6.1)
Cr (μmol/L) (median, IQR)	83.0 (71.2–97.1)
UA (μmol/L) (median, IQR)	315.0 (259.6–379.6)
TG (mmol/L) (median, IQR)	1.5 (1.2–2.1)
TC (mmol/L) (median, IQR)	4.4 (3.6–5.2)
HDLC (mmol/L) (median, IQR)	1.0 (0.9–1.2)
LDLC (mmol/L) (median, IQR)	2.7 (2.1–3.3)
Hcy (μmol/L) (median, IQR)	13.2 (11.1–16.5)
Fib (g/L) (median, IQR)	3.3 (2.8–4.0)
TSH (uIU/mL) (median, IQR)	1.8 (1.2–3.0)
FT3 (pmol/L) (median, IQR)	4.0 (3.5–4.4)
FT4 (pmol/L) (median, IQR)	15.4 (13.5–17.6)
NIHSS score at admission (median, IQR)	4 (2–8)

Abbreviations: sICAS, symptomatic intracranial atherosclerosis; DM, diabetes mellitus; CAD, coronary artery disease; TIA, transient ischemic attacks; WBC, white blood cell; PLT, platelet; NLR, neutrophil lymphocyte ratio; AGR, albumin/globulin ratio; TBIL, total bilirubin; TBA, total bile acid; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; TG, triglyceride; TC, total cholesterol; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; Hcy, homocysteine; Fib, fibrinogen; TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine 4; NIHSS, the National Institutes of Health Stroke Scale.

Different Stenosis Characteristics Between Early-Onset and Late-Onset sICAS Groups

We presented the discrepancy of stenosis distribution in Figure 2. The location of the stenosis between the two groups showed no significant difference neither in the anterior nor in the posterior cerebral circulation (p=0.257). Stenosis tends to be located in the anterior circulation of the two groups (67.4% in the early-onset group vs 69.8% in the late-onset group). When compared with the late-onset group, the early-onset group had a lower number of stenosis, despite no apparent statistical significance between the two groups (p=0.693). Moreover, severe vascular stenosis was presented in the early-onset sICAS group when compared with the late-onset group (severe stenosis: 23.8% in the early-onset group vs 20.0% in the late-onset group; occlusion: 39.5% in the early-onset group vs 38.2% in the late-onset group; p=0.006).

Association Between Molecular Biomarkers and sICAS in Both Early-Onset and Late-Onset Group

We performed a univariate analysis to compare risk factors and plasma molecular biomarkers between groups. As presented in Table 2, male, smoking, drinking, dyslipidemia and high levels of white blood cell (WBC), platelet (PLT), albumin globulin ratio (AGR), triglyceride (TG), free triiodothyronine (FT3) were more frequent in the early-onset group ($p<0.05$), while high frequency of hypertension, coronary artery disease (CAD), high levels of neutrophil lymphocyte ratio (NLR), total bile acid (TBA), blood urea nitrogen (BUN), high-density lipoprotein cholesterol (HDLC), homocysteine (Hcy) and fibrinogen (Fib) were found in the late-onset group ($p<0.05$). No significant differences were found when referred to the history of stroke, diabetes, the levels of total bilirubin (TBIL), serum creatinine, total cholesterol, low-density lipoprotein and thyroid-stimulating hormone (TSH) between the two groups. The severity of stroke at admission was assessed using the National Institutes of Health Stroke Scale. We found a lower NIHSS score in the early-onset group (median, 4; IQR, 2–8) than in the late-onset group (median, 5; IQR, 2–8), while it showed no statistical difference after univariate analysis (p=0.062).

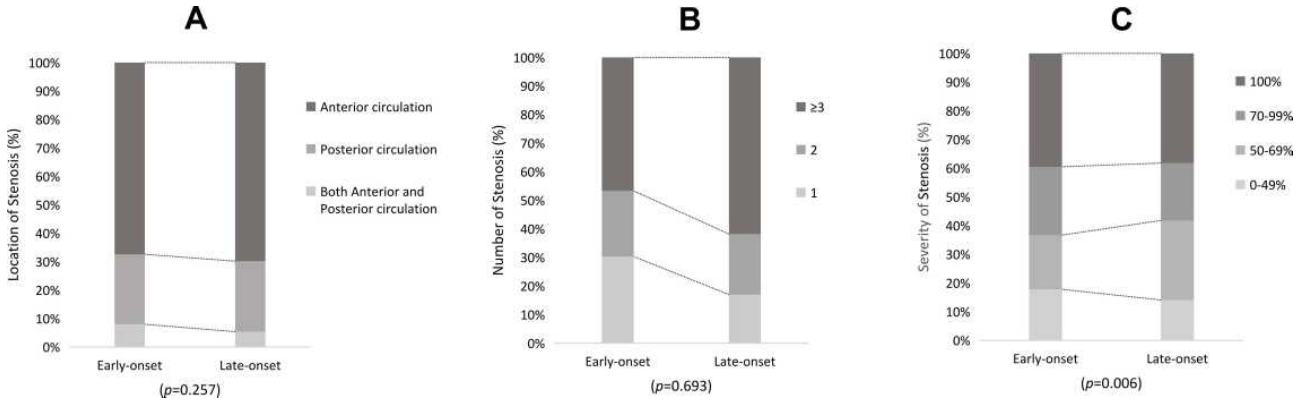

Figure 2 Different stenosis characteristics between early-onset and late-onset sICAS group (**A-C**).
Notes: Early-onset sICAS group defined as age ≤60 years old and late-onset sICAS group defined as age >60 years old; P-values <0.05 were considered statistically significant.

Logistic Regression Analyses Reveal the Independent Biomarkers Associated with the Early-Onset sICAS

Statistically significant variables showing p<0.05 in the univariate analysis were selected as independent variables of the binary logistic regression analysis. As shown in Figure 3, a higher level of white blood cells (Adjusted OR, 95% CI: 2.060 (1.360–3.120); p=0.001), PLT (Adjusted OR, 95% CI: 1.567 (1.046–2.348); p=0.029), AGR (Adjusted OR, 95% CI: 2.060 (1.205–2.680); p=0.004) and FT3 (Adjusted OR, 95% CI: 1.869 (1.259–2.775); p=0.002), also a lower level of TBA (Adjusted OR, 95% CI: 0.576, (0.391–0.849); p=0.005), BUN (Adjusted OR, 95% CI: 0.573 (0.390–0.840); p=0.004), HDLC (Adjusted OR, 95% CI: 0.621 (0.417–0.926); p=0.020), Hcy (Adjusted OR, 95% CI: 0.485 (0.321–0.733); p=0.001) and Fib (Adjusted OR, 95% CI: 0.572 (0.381–0.859); p=0.007) were independent risk factors of early-onset sICAS group after adjusting for the sex, stroke history, hypertension, coronary heart disease, diabetes, dyslipidemia, family history of stroke, smoking and drinking.

Discussion

Evidence focusing on biomarkers of early-onset sICAS is limited. In this study, we found that higher baseline levels of WBC, PLT, FT3, AGR and lower levels of TBA, BUN, HDLC, Hcy, and Fib were independent predictors of early-onset sICAS.

Recent study has demonstrated that the WBC level in younger patients with the symptomatic intracranial atherosclerotic disease increases,[22] which is in line with our study. As an inflammatory biomarker, WBC was associated with early atherosclerosis and late plaque formation in carotid artery diseases.[23] WBC plays a crucial role in initiating and spreading atherosclerosis through rolling, adhesion, migration and activation.[24] Activated WBCs tend to adhere to vascular endothelial cells and penetrate the intima, resulting in capillary leukocytosis and vascular resistance.[25] Furthermore, WBCs release various hydrolases, cytokines and growth factors, leading to endothelial dysfunction, arterial elastic properties alteration and structural stiffness.[25]

Platelet also has an impact on the early stage of atherosclerotic injury.[26,27] When the vascular injury occurs, the ruptured plaques initiate a series of responses involving platelet adhesion, platelet activators release, platelet morphological modification and subsequent granular contents release, and finally the glycoprotein IIb/IIIa receptors activation and the thrombus reaction enhancement.[28] Various receptors markedly affect the firm platelet adhesion to the endothelial extracellular matrix, including glycoprotein receptors GPIb/IX/V, GPVI, GPIb, GPIIb/IIIa and collagen receptor a2b1.[29] Activated platelets can further release a big heap of inflammatory mediators, inducing the chemotaxis, adhesion, and transmigration of the leukocytes to the inflammatory site,[30] and promote the leukocyte recruitment and inflammatory response by morphology change, reactive oxygen species (ROS) production, neutrophil extracellular traps formation.[23,31,32] Specific interactions with platelet-leukocyte may provide a therapeutic method for preventing early atherosclerotic lesions.[14,24]

The relationship between thyroid hormone and atherosclerosis is controversial. Some studies showed that thyroid hormones can stimulate atherosclerosis, while other studies indicated that thyroid hormones can inhibit atherosclerosis progression by regulating vasodilatation,

Table 2 Comparison of Demographic, Laboratory and Clinical Features in Patients with Early and Late-Onset sICAS

	Early-Onset (≤60) n=488	**Late-Onset (>60) n=519**	**P-value**
Sex (male, N, %)	343 (70.3%)	302 (58.2%)	<0.001
Smoking (N, %)	247 (50.6%)	197 (38.0%)	<0.001
Drinking (N, %)	187 (38.3%)	147 (28.3%)	0.001
History			
Stroke History (N, %)	95 (19.5%)	109 (21.0%)	0.545
Hypertension (N, %)	344 (70.5%)	425 (81.9%)	<0.001
DM (N, %)	168 (34.4%)	172 (33.1%)	0.666
Dyslipidemia (N, %)	258 (52.9%)	219 (42.2%)	0.001
CAD (N, %)	50 (10.2%)	100 (19.3%)	<0.001
Family History of Stroke (N, %)	29 (5.9%)	22 (4.2%)	0.218
Ischemic type			0.223
Stroke (N, %)	458 (93.9%)	496 (95.6%)	
TIA (N, %)	30 (6.1%)	23 (4.4%)	
WBC ($\times 10^9$/L) (median, IQR)	7.0 (5.7–8.5)	6.5 (5.4–8.0)	0.002
PLT ($\times 10^9$/L) (median, IQR)	212.0 (172.2–257.0)	195.0 (161.0–239.0)	<0.001
NLR (median, IQR)	2.5 (1.9–3.5)	2.8 (2.0–4.2)	0.008
AGR (median, IQR)	1.5 (1.4–1.7)	1.4 (1.2–1.6)	<0.001
TBIL (μmol/L) (median, IQR)	9.9 (7.2–12.6)	9.9 (7.6–13.7)	0.181
TBA (μmol/L) (median, IQR)	3.1 (2.0–4.9)	3.8 (2.4–6.6)	<0.001
BUN (mmol/L) (median, IQR)	4.7 (3.7–5.7)	5.3 (4.2–6.3)	<0.001
Cr (μmol/L) (median, IQR)	82.1 (71.0–94.0)	83.0 (71.7–98.0)	0.194
UA (μmol/L) (median, IQR)	316.6 (264.0–371.6)	310.8 (254.5–386.6)	0.607
TG (mmol/L) (median, IQR)	1.7 (1.2–2.3)	1.4 (1.1–1.9)	<0.001
TC (mmol/L) (median, IQR)	4.3 (3.5–5.2)	4.4 (3.6–5.2)	0.188
HDLC (mmol/L) (median, IQR)	1.0 (0.8–1.2)	1.1 (0.9–1.3)	<0.001
LDLC (mmol/L) (median, IQR)	2.6 (2.1–3.3)	2.7 (2.1–3.3)	0.595
Hcy (μmol/L) (median, IQR)	12.6 (10.7–15.2)	13.7 (11.3–17.6)	<0.001
Fib (g/L) (median, IQR)	3.2 (2.7–3.9)	3.4 (2.8–4.2)	0.001
TSH (mIU/mL) (median, IQR)	1.8 (1.2–2.9)	1.8 (1.2–3.1)	0.375
FT3 (pmol/L) (median, IQR)	4.1 (3.6–4.6)	3.9 (3.4–4.3)	<0.001
FT4 (pmol/L) (median, IQR)	15.5 (13.6–17.6)	15.4 (13.4–17.6)	0.899
NIHSS score at admission (median, IQR)	4 (2–8)	5 (2–8)	0.062

Notes: P-values <0.05 were considered statistically significant.

macrophage function, and VSMC proliferation.[33–35] Clinical evidence showed a correlation between the elevated FT3 level and the risk of subclinical and clinical atherosclerosis,[36] indicating that increased thyroid hormones may lead to atherosclerosis. The role of FT3 in early-onset sICAS may involve the following three

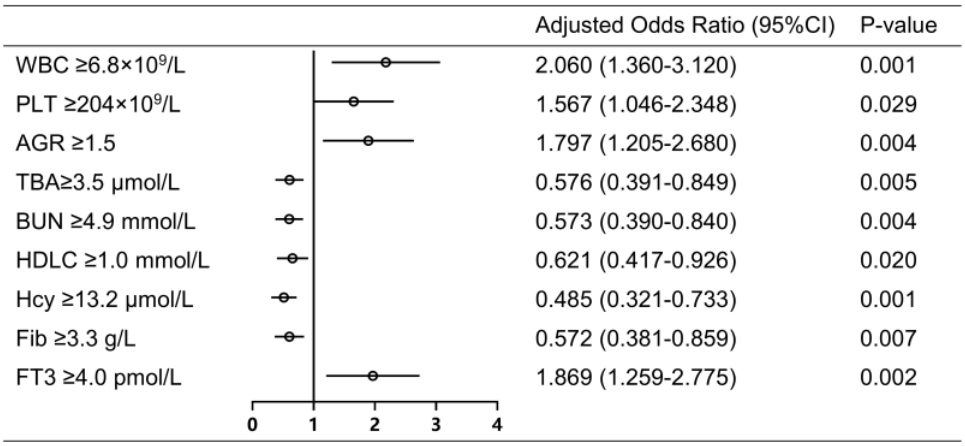

Figure 3 Logistic regression analyses for factors independently associated with the early-onset sICAS.
Notes: The cut-off value for each biomarker was determined by the median. P-values <0.05 were considered statistically significant.

aspects: First, excessive thyroid hormone can accelerate the release of ROS, induce the adhesion to the endothelial cell, and lead to endothelial dysfunction. Second, thyroid hormone regulates the synthesis of procoagulant proteins and promotes plaque vulnerability and rupture. Third, thyroid hormone increases cardiac load and myocardial oxygen consumption, eventually leading to ischemic events and death.[36] What's more, elevated level of antithyroid peroxidase antibody (TPO-Ab) was found in young (age ≤55 years old) stroke patients with intracranial stenosis,[37] and TPO-Ab could induce ICAS by promoting the migration of vascular smooth muscle cells.[38] However, in our data, TPO-Ab level was excluded from the final analysis owing to a substantial number of missing values. The available data showed that patients in early-onset group had higher mean concentrations of TPO-Ab than those in the late group (44.92 vs 35.43 U/mL). Further research should be conducted to confirm this issue.

In addition to the above-mentioned indicators, we found that the level of AGR was higher in the early-onset group, that is, AGR was lower in late-onset sICAS patients. AGR refers to the ratio of plasma albumin to non-albumin protein, while non-albumin includes fibrinogen, prothrombin, inflammatory proteins and other regulatory proteins except globulin.[39] Thus, not only the increased plasma albumin concentration but also the decreased fibrinogen level plays a crucial role in the high AGR ratio, which is consistent with our findings that lower fibrinogen level was found in the early-onset sICAS group. Clinical evidence has shown that low AGR has an adverse impact on atherosclerosis and cardiocerebrovascular events.[39,40] Due to the increased aging associated with malnutrition and inflammatory reaction, albumin is decreasing in the elderly.[39,40] High level of AGR might be just a biomarker of early-onset sICAS, while lower level of AGR and albumin may be risk factors for late-onset sICAS.

Besides, a reduced plasma level of TBA, BUN, HDLC, and Hcy was also found in the early-onset sICAS group. Previous studies have revealed that excessive cholesterol converts into bile acids, then discharges from feces in the form of bile salt,[41] while HDLC transports cholesterol back to the liver from the outside in reverse transportation.[42] All of the above results indicated that low levels of HDLC and TBA were risk factors for early sICAS. High level of BUN was considered to be associated with severe limb ischemia.[43] Clinical evidence found that elevated Hcy accelerated atherosclerosis through monocyte activation, endothelial cell injury, lipid synthesis disorder, stimulation of VSMC proliferation and thrombosis.[44] Although not well-understood for the lower levels of BUN and Hcy in the early-onset group than in the late-onset group, age may play a role leading to the decrease of these indicators. These findings indicate different pathophysiological mechanisms between early- and late-onset sICAS.

There are some limitations in our study. First, this study adopted a single-center clinical sample, and most patients lived in the central area of the Hunan province, resulting in a regional difference. Second, since it is a cross-sectional study, it is impossible to draw a causal inference between the early-onset sICAS and the risk factors. Third, the baseline plasma or serum biomarkers were collected at admission; however, it actually should be

a dynamic process. Fourth, the number of TIA patients was relatively small, so we need to expand the sample size in the future. Fifth, tools for measuring ICAS were not unified with MRA/CE-MRA/CTA/DSA/, most patients were evaluated by MRA and CE-MRA, which may exaggerate the stenosis of intracranial arteries. Finally, early-onset asymptomatic ICAS individuals were not included in this study, which is also of major importance.

Our research also has several strengths. First, this was a big clinical study with large sample size from Xiangya Hospital and included 1007 patients, which greatly improved the statistical confidence and reduced the random errors. Second, the molecular indicators obtained from the clinical serum samples of patients have the advantages of easy detection and low cost. Third, the mechanisms for early-onset intracranial atherosclerosis have not been reported previously, and there have been no clinical trials investigating the molecular index differences between the early and late-onset sICAS groups. Finally, our results suggest that the combined detection of these molecular indicators has a positive significance for identifying patients with a higher risk of early-onset sICAS, and close follow-up should be emphasized for these patients. The prognostic value and potential therapeutic effect of these molecular indicators are worth further study in the future.

Conclusion

In conclusion, high levels of WBC, Plt, AGR and FT3, as well as low levels of TBA, BUN, HDLC, Hcy and Fib are associated with early-onset sICAS when compared with late-onset sICAS. Detection of these molecular biomarkers may provide a simple way for the prevention and diagnosis of early sICAS. Identification of additional biomarkers associated with early-onset ICAS, either symptomatic or asymptomatic, should be discussed in the future.

Ethics Approval and Informed Consent

Written informed consent was obtained from each patient included in the study, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been priorly approved by the Ethics Committee of Xiangya Hospital of Central South University, China.

Consent for Publication

We confirm that the details of any images, videos, recordings, etc., can be published, and that the person(s) providing consent have been shown the article contents to be published.

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Author Contributions

J.X. and F.Y. designed the study. Y.L. wrote the manuscript. F.Y. and Y.L. performed the analysis and interpretation of data as well as the manuscript revision. X.F., D.L., M.W., X.L., Q. H., Z.L., L.Z., T.Z., and R.T. were responsible for the acquisition and interpretation of data. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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