Original Article

Plasma RANTES level is correlated with cardio-cerebral atherosclerosis burden in patients with ischemic cerebrovascular disease

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Abstract

Background: Regulated upon activation, normal T-cell expressed, and secreted (RANTES) is a chemokine actively involved in the initiation and progression of atherosclerosis (AS), which is the major cause of ischemic cerebrovascular disease (ICVD). This study aimed to determine the associations between circulating RANTES level and overall AS conditions of cardiac and cerebral vessel beds in patients with ICVD.

Methods: Patients with ICVD admitted to the department of neurology of Xuanwu Hospital from April 1, 2019 to June 30, 2019 were prospectively enrolled in the study. Plasma RANTES level was measured by enzyme-linked immunosorbent assay to represent the circulating RANTES level. The integrated AS burden of the cervicocephalic and coronary arteries was examined using computed tomography angiography and reflected by "cardio-cerebral AS burden (CCAB)" as a continuous variable. Then, the relationship of plasma RANTES level and CCAB in patients with ICVD was analyzed by correlation analyses and general linear models.

Results: A total of 40 patients with ICVD were included in the study. There was a significant positive correlation between CCAB and plasma RANTES level in ICVD ($r = 0.786$, $P < 0.001$), independent of age, sex, acute or chronic phase of ICVD, and mono or dual antiplatelet therapy (adjusted B for ln RANTES, 12.063; 95% confidence interval, 7.572−16.533). The association of plasma RANTES level with AS conditions (burden, severity, and extent) in single cardiac or cerebral vessel bed was similar to that with CCAB, but the correlation coefficient for CCAB was higher (increment ranged from 0.126 to 0.397).

Conclusions: Plasma RANTES level was an independent indicator for the integrated AS burden of the cervicocephalic and coronary arteries in ICVD. Comprehensive evaluation of AS conditions using the novel continuous index CCAB might be

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Peer review under responsibility of Chinese Medical Association.

important in revealing the systematic relationship between circulating RANTES and AS in patients with ICVD.

Introduction

Atherosclerosis (AS) is the leading cause of ischemic cerebrovascular disease (ICVD).[1] As a chronic inflammation, the initiation and progression of AS is closely associated with chemokines, which direct leukocytes to the inflammatory tissues.[2] Regulated upon activation, normal T-cell expressed, and secreted (RANTES) is one of the most well-studied AS-related chemokines.[3] It can be synthesized and secreted by platelets, $CD8^+$ T cells, endothelial cells, fibroblast cells, and other cells.[4] RANTES has been recognized as an important link between platelets and leukocytes, both of which are overactivated in patients with ICVD and actively participate in the process of atherogenesis.[5] RANTES is present in both peripheral blood and atherosclerotic plaques,[4] and its circulating and tissue levels were found to be well correlated.[6]

Animal studies had demonstrated that circulating RANTES could be deposited on the surface of vascular endothelial cells, facilitating the recruitment of leukocytes to atherosclerotic plaques and increasing lesion volume.[7] Additionally, blocking the receptor of RANTES significantly reduced the degree of AS in mice.[8,9] However, the associations between human circulating RANTES level and AS conditions are still controversial. There was evidence that circulating RANTES level was positively related to the volume of carotid plaques in the general population,[10] and higher circulating RANTES level had been shown to be predictive for the presence of coronary stenosis $\geq 50\%$ in patients undergoing coronary angiography.[11] However, Podolec et al[12] found that the RANTES level was lower in patients with more diffuse coronary stenosis $\geq 50\%$. Apart from distinct study populations, different samples for testing RANTES level and different methods for assessing AS conditions could also contribute to the discrepancy in the abovementioned studies.

Generally, serum and plasma RANTES levels were measured to reflect the circulating RANTES level in humans.[4,9–11] A previous study focused on the relationship of circulating RANTES level and AS conditions in patients with ICVD using serum samples.[13] However, RANTES would be further released by platelets during the clotting process when the serum sample was generated from peripheral venous blood *ex vivo*.[4] Therefore, serum RANTES level might fail to accurately reflect the circulating RANTES level, whereas plasma RANTES level could be a more reliable alternative.

Furthermore, previous studies evaluated only AS conditions in the localized artery or single vessel bed, without paying attention to the integrated AS conditions in multiple arteries.[9–11] Considering that RANTES could exert systematic influences on all arteries through the circulation and AS often simultaneously develops in multiple vessel beds,[14] it would be appropriate to assess the AS conditions in a more comprehensive manner to explore the associations between circulating RANTES and AS. The "stenosis severity score" summarizes the severity scores of AS in various coronary arterial segments to represent the overall AS conditions in coronary arteries, which had been proved to independently predict prognosis.[15] However, there had been no study on this method to simultaneously evaluate cervicocephalic and coronary arteries to reflect the "cardio-cerebral AS burden (CCAB)". Theoretically, the novel continuous index CCAB could be a more systematic representation of the AS conditions of patients with ICVD and would manifest the correlations of circulating RANTES and AS more quantitatively compared to categorical indices.

Therefore, we measured the plasma RANTES level of patients with ICVD and examined their cervicocephalic and coronary arteries simultaneously by computed tomography angiography (CTA), assessing the integrated AS conditions of these two vessel beds. This study aimed to determine the associations between plasma RANTES level and CCAB in patients with ICVD.

Methods

Ethical approval

This was a single-center cross-sectional study approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University (No. 2018065). All procedures were in accordance with the *Declaration of Helsinki*, and all participants provided written informed consent.

Study subjects

Patients with ICVD admitted to the department of neurology of Xuanwu Hospital from April 1, 2019 to June 30, 2019 were enrolled consecutively. The inclusion criteria were as follows: ICVD with acute cerebral infarction or transient ischemic attack with symptom onset within 90 days; age ≥45 years; at least two risk factors (history of hypertension, history of diabetes, history of hyperlipidemia, smoking, overweight [body mass index ≥ 25 kg/m²], and history of coronary heart disease); mono or dual antiplatelet therapy; appropriateness for CTA; and written informed consent. The exclusion criteria were as follows: non-atherosclerotic stenosis, such as arterial dissection, fibromuscular dysplasia, and vasculitis; intracranial hemorrhage; infection, hematologic disease, and immune disease; use of immunomodulatory drugs, such as hormones and immunosuppressants; use of subcutaneous or oral anticoagulants; and severe organ dysfunction, malignant tumor, pregnancy, or nondiagnostic image quality (see below).

General characteristics

Data on age, sex, symptom onset time, height, weight, smoking status, history of hypertension, history of diabetes, history of hyperlipidemia, and history of coronary heart disease were collected personally using a special form. The systolic and diastolic blood pressures at admission and antiplatelet treatment were recorded. On the second day after admission, blood examinations, such as routine, biochemical, and coagulation tests, were performed. White blood cell counts, platelet counts, and low-density lipoprotein cholesterol, fasting blood glucose, fibrinogen, D-dimer, and high-sensitivity C-reactive protein levels were recorded.

Evaluation of CCAB

The scans were performed using a dual-source 192-slice computed tomography scanner (Somatom Force, Siemens Healthcare, Forchheim, Germany). The amount of contrast material (Ultravist 370 mg iodine/mL; Bayer Schering Pharma, Germany) was adjusted according to the body mass index of the patient and ranged between 40 and 50 mL. All CTA datasets were reconstructed with a slice thickness of 0.6 mm and an increment of 0.4 mm using a medium smooth reconstruction kernel (Bv36) and transferred to an external workstation (MMWP, syngo.via, Siemens, Germany). Curved planar reformatting, maximum intensity projection, multiplanar reformatting, and volume rendering images were used to evaluate the coronary and cervicocephalic arteries. The percentage of arterial stenosis was quantified on orthogonal views with an automatic vessel analysis tool using the North American Symptomatic Carotid Endarterectomy Trial method[16] for the cervical arteries and Warfarin-Aspirin Symptomatic Intracranial Disease Study Trial method[17] for the intracranial and coronary arteries. An intention-to-diagnose strategy would be applied if the stenosis degree of one arterial segment was uninterpretable, while the image quality would be considered as nondiagnostic and the patient would be excluded from the final analysis if the stenosis degree of more than one arterial segment was uninterpretable.[18] Two certified radiologists blinded to the clinical data reviewed the angiographies independently, and the discrepancy was settled by discussion. The kappa coefficient for the initial judgments of arterial stenosis degree by the two radiologists was 0.72 based on 260 arterial segments in 10 patients.

A total of 21 segments of the cervicocephalic arteries (bilateral) were evaluated, including subclavian, common carotid, extracranial internal carotid, external carotid, extracranial vertebral, intracranial internal carotid, intracranial vertebral, basilar, anterior cerebral, middle cerebral, and posterior cerebral arteries. Moreover, 5 coronary arterial segments were observed, including the left main trunk, anterior descending branch, diagonal branch, circumflex branch, and right coronary artery. The AS severity score ranged from 0 to 4 points: 0, no atherosclerotic plaque or any degree of arterial stenosis; 1, stenosis <50%; 2, stenosis of 50%–69%; 3, stenosis of 70%–99%; 4, arterial occlusion. CCAB was calculated by summing the AS severity scores of all observed cervicocephalic and coronary arterial segments.

To reflect the AS conditions in single cardiac or cerebral vessel bed, the AS severity, extent, and burden were measured. AS severity was represented by the most severe stenosis in the cardiac or cerebral vessel bed, while AS extent was defined as the number of cervicocephalic or coronary arterial segments with stenosis ≥50%. AS burden was the sum of AS severity scores of all observed cervicocephalic or coronary arterial segments.

Measurement of plasma RANTES level

Fasting peripheral venous blood (2 mL) was collected with ethylenediaminetetraacetic acid anticoagulant in the morning. The platelet-poor plasma was obtained within 30 min by double centrifugation

(1000 g × 15 min + 10000 g × 10 min) at 4 °C. The RANTES level in platelet-deficient plasma was measured according to the instructions of the enzyme-linked immunosorbent assay kit (catalog number DRN00B, R&D Systems, USA). Each sample was repeatedly tested, and the average level was recorded as the final plasma RANTES level.

Statistical analysis

Statistical analyses were performed using SPSS (version 25.0, IBM, USA) and MedCalc (version 15.0, MedCalc Software, Belgium). Data were presented as mean ± standard deviation for normally distributed continuous variables, count (with percentage) for dichotomous variables, and medians (Q1, Q3) for abnormally distributed continuous and ordinal variables. Student's *t*-test was used for normally distributed continuous variables, Mann−Whitney *U* test was used for abnormally distributed continuous variables and ordinal variables, and chi-square test was used for dichotomous variables. Bivariate correlation analysis was used to evaluate the correlation between plasma RANTES level and CCAB. Pearson correlation analysis was used for normally distributed continuous variables. Spearman correlation analysis was used for abnormally distributed continuous and ordinal variables. With appropriate variable transformations, the independent associations between plasma RANTES level and CCAB were analyzed using general linear models with adjustment for age, sex, onset of ICVD within ≥14 days, dual or mono antiplatelet therapy, and all general characteristics, which were significantly different ($P < 0.05$) between patients with ICVD with CCAB in the highest and lowest tertiles. Adjusted *B*-value was calculated with 95% confidence interval (CI). A *P* value < 0.05 was considered statistically significant.

Results

General characteristics of included patients with ICVD

A total of 40 patients with ICVD were included in this study, with an average age of 62 ± 8 years (minimum, 46 years; maximum, 77 years). Among these, 82.5% were male, 50% had the onset of ICVD within 14 days, and 52.5% received dual antiplatelet therapy. The median plasma RANTES level was 33449 pg/mL (Q1 25446 pg/mL, Q3 49332 pg/mL), and the average

CCAB was 20 ± 8 points (minimum, 6 points; maximum, 37 points).

Relationship of plasma RANTES level and cardiac and cerebral AS conditions in ICVD

According to the bivariate correlation analysis, plasma RANTES level was positively associated with CCAB ($r = 0.786$, $P < 0.001$). This relationship was consistent with the correlations between plasma RANTES level and AS conditions (burden, severity, and extent) of single cardiac or cerebral vessel bed, but the correlation coefficient tended to be higher for CCAB (Table 1).

CCAB and log transformation of plasma RANTES level (*ln* RANTES) were normally distributed, and the scatter plot suggested that *ln* RANTES had a linear association with CCAB (Fig. 1). Comparing the general characteristics of patients with ICVD with CCAB in the highest ($n = 14$) and lowest tertile ($n = 14$), only age was significantly different between the two groups (66.6 ± 5.6 years vs. 59.4 ± 7.1 years, $P = 0.006$) (Table 2). Then, variables, including age, sex, onset of ICVD within ≥14 days, and dual or mono antiplatelet therapy, were entered into a general linear model to examine the independent effects of plasma RANTES level on CCAB as preplanned. It was found that *ln* RANTES was positively correlated with CCAB independent of the abovementioned factors (adjusted $B = 12.063$; 95% CI, 7.572−16.533).

We further tested whether the associations between plasma RANTES level and CCAB would vary with the distribution of atherosclerotic lesions in patients with ICVD. The plasma RANTES level was still significantly positively correlated with CCAB, among either patients with ICVD with AS ≥50% in both cardiac and cerebral vessel beds ($n = 19$, $r = 0.762$, $P < 0.001$) or those with AS ≥50% in only one of the two vessel beds ($n = 21$, $r = 0.785$, $P < 0.001$).

Additionally, we also observed the associations between plasma RANTES level and AS conditions that were dichotomously assessed in patients with ICVD. The presence of multiple stenosis ≥50% in cervicocephalic arteries ($n = 31$), coronary arteries ($n = 10$), and both cervicocephalic and coronary arteries ($n = 19$) were all related to a significantly higher plasma RANTES level (Table 3). However, whether there was any AS or stenosis ≥50% in cervicocephalic arteries and whether there was any AS or stenosis ≥50% in coronary arteries had no obvious relationship with plasma RANTES level ($P > 0.05$).

Table 1
Bivariate correlation analysis between plasma RANTES level and AS conditions of cardiac and cerebral vessel beds in patients with ICVD.

Variables	Plasma RANTES			
	Correlation coefficient[a]	P-value for correlation coefficient	Changes in correlation coefficient compared with that of CCAB	
			Z-value	P
CCAB	0.786	<0.001[e]	−	
Cerebral AS burden[b]	0.649	<0.001[e]	1.236	0.2170
Cardiac AS burden[b]	0.520	0.001[e]	2.084	0.0372[e]
Cerebral AS severity[c]	0.389	0.013[e]	2.797	0.0052[e]
Cardiac AS severity[c]	0.460	0.003[e]	2.424	0.0154[e]
Cerebral AS extent[d]	0.660	<0.001[e]	1.153	0.2490
Cardiac AS extent[d]	0.416	0.008[e]	2.658	0.0079[e]

ICVD: ischemic cerebrovascular disease; RANTES: regulated upon activation, normal T-cell expressed, and secreted; CCAB: cardio-cerebral atherosclerosis burden; AS: atherosclerosis.

[a] Pearson correlation analysis was used for normally distributed continuous variables. Spearman correlation analysis was used for abnormally distributed continuous variables and ordinal variables.

[b] Cerebral or cardiac AS burden was calculated as the sum of AS severity scores (range, 0−4 points: 0, no atherosclerotic plaque or any degree of arterial stenosis; 1, stenosis <50%; 2, stenosis of 50%−69%; 3, stenosis of 70%−99%; 4, arterial occlusion) of each segment in cervicocephalic or coronary arteries, respectively.

[c] Cerebral or cardiac AS severity was the severity score of the most severe stenosis in cervicocephalic or coronary arteries, respectively.

[d] Cerebral or cardiac AS extent was defined as the number of cervicocephalic or coronary arterial segments with stenosis ≥50%, respectively.

[e] A P-value <0.05 was considered statistically significant.

General characteristics associated with plasma RANTES level in ICVD

Plasma RANTES level was divided into three, and the general characteristics of patients with ICVD in the highest ($n = 13$) and lowest tertile ($n = 13$) were compared. A history of hypertension was more frequent in patients with ICVD with the upper tertile of plasma RANTES level (100% *vs.* 69.2%, $P = 0.012$), while other general characteristics were not significantly different between the two groups (Table 4).
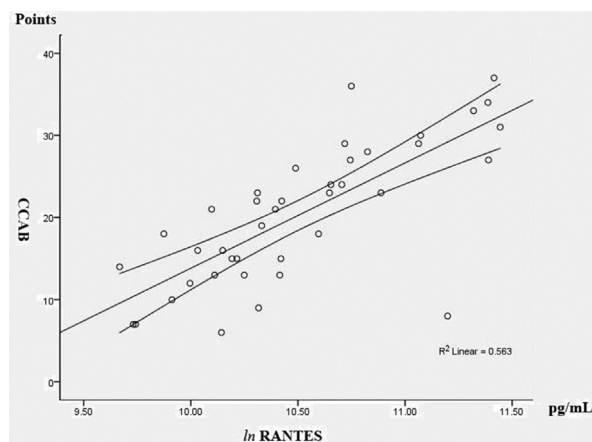

Fig. 1. Scatter plot of *ln* RANTES and CCAB in patients with ICVD. ICVD: ischemic cerebrovascular disease; CCAB: cardio-cerebral atherosclerosis burden; RANTES: regulated upon activation, normal T-cell expressed, and secreted.

Discussion

In this study, both cervicocephalic and coronary arteries were evaluated by CTA; then, a continuous variable CCAB was obtained to more comprehensively reflect the AS conditions for patients with ICVD, and the RANTES level in plasma rather than serum was tested to more accurately reflect the circulating RANTES level. The results showed a significant and independent positive correlation of CCAB and plasma RANTES level in ICVD ($r = 0.786$), being consistent with the associations between plasma RANTES level and AS conditions (burden, severity, and extent) of single cardiac or cerebral vessel bed but having a higher degree of correlation (differences of correlation coefficient ranged from 0.126 to 0.397).

There had been some studies on the relationship of serum RANTES level with the components of atherosclerotic plaques in the internal carotid artery and the risk of cardiocerebrovascular events in patients with symptomatic internal carotid arterial stenosis,[13] but the relevance of plasma RANTES level to AS severity and extent in patients with ICVD was still unexplored. A previous magnetic resonance study on carotid plaques in the general population manifested that plasma RANTES level was positively correlated with carotid wall and lipid-core volumes, as well as maximum wall and fibrous cap thicknesses. Therefore, it was believed that higher plasma RANTES level might indicate more

Table 2
General characteristics of patients with ICVD with the highest and lowest tertiles of CCAB.

Characteristics	Total ($n = 40$)	Highest tertile ($n = 14$)	Lowest tertile ($n = 14$)	Statistical values	P
Age (years)	62 ± 8	67 ± 6	60 ± 7	−2.983[a]	0.006[d]
Male	33 (82.5)	12 (85.7)	12 (85.7)	0.000[b]	1.000
Hypertension history	31 (77.5)	13 (92.9)	9 (64.3)	3.642[b]	0.056
Diabetes mellitus history	18 (45.0)	6 (42.9)	5 (35.7)	0.150[b]	0.699
Hyperlipidemia history	33 (82.5)	13 (92.9)	10 (71.4)	2.320[b]	0.128
Coronary artery disease history	18 (45.0)	9 (64.3)	6 (42.9)	1.292[b]	0.256
Current smoking	14 (35.0)	6 (42.9)	6 (42.9)	0.000[b]	1.000
Overweight	22 (55.0)	10 (71.4)	5 (35.7)	3.590[b]	0.058
Onset of ICVD within 14 days	20 (50.0)	7 (50.0)	7 (50.0)	0.000[b]	1.000
Dual antiplatelet therapy	21 (52.5)	7 (50.0)	6 (42.9)	0.144[b]	0.705
SBP at admission (mmHg)	144.1 ± 24.2	143.9 ± 31.5	144.4 ± 23.8	0.047[a]	0.963
DBP at admission (mmHg)	83.6 ± 14.9	80.1 ± 15.9	86.7 ± 16.1	1.098[a]	0.282
Leukocyte number (10^9/L)	6.97 ± 2.18	6.11 ± 1.63	7.59 ± 2.73	1.746[a]	0.093
Platelet number (10^9/L)	207 (176, 227)	202 (171, 226)	210 (168, 225)	−0.276[c]	0.783
Fasting glucose (mg/dL)	5.12 (4.56, 6.52)	5.35 (4.64, 6.64)	4.72 (4.42, 7.35)	−0.388[c]	0.698
LDL-C (mmol/L)	1.87 (1.62, 2.37)	1.81 (1.43, 2.32)	2.11 (1.74, 2.96)	−1.335[c]	0.182
Fibrinogen (g/L)	3.43 ± 0.76	3.25 ± 0.52	3.61 ± 1.03	1.098[a]	0.249
D-dimer (mg/L)	0.28 (0.17, 0.45)	0.30 (0.20, 0.58)	0.27 (0.14, 0.38)	−1.093[c]	0.274
hsCRP (mg/L)	1.15 (0.42, 2.79)	0.90 (0.41, 1.61)	1.43 (0.37, 5.13)	−0.688[c]	0.491

Data were shown as mean ± SD, median (Q1, Q3), or n (%).
ICVD; ischemic cerebrovascular disease; CCAB; cardio-cerebral atherosclerosis burden; SBP; systolic blood pressure; DBP; diastolic blood pressure; LDL-C; low-density lipoprotein cholesterol; hsCRP; hypersensitive C-reactive protein.
[a] t values.
[b] Chi-square value.
[c] Z value.
[d] A P-value <0.05 was considered statistically significant.

active inflammation and fibroproliferation in plaques.[10] Similarly, our results suggested that the increase in plasma RANTES level was associated with the increase in CCAB in patients with ICVD. It not only echoed previous findings of related animal experiments[6−8] but also further supported the notion that RANTES could actively participate in the initiation and progression of AS.

More interestingly, this study showed that the positive correlation of plasma RANTES level and CCAB existed independent of factors such as age, sex, acute or chronic phase of ischemic events, and strength of antiplatelet therapy (adjusted B for ln RANTES, 12.063; 95% CI, 7.572−16.533). There had been studies demonstrating that these factors could significantly affect circulating RANTES level,[10,19−21] but we found that these factors would not obviously change the relationship between plasma RANTES level and CCAB. Thus, the plasma RANTES level might serve as a stable marker of integrated AS conditions of cardiac and cerebral vessel beds in patients with ICVD, but more studies with larger sample size would be needed to confirm these findings.

Based on the close relationship between AS conditions of cardiac and cerebral vessel beds,[22] we summed the AS severity scores of all segments of cervicocephalic and coronary arteries and generated CCAB, a continuous variable with large range (0−104 points), to systematically reflect the cardiac and cerebral AS conditions. Compared with just assessing AS conditions of single cardiac or cerebral vessel bed, CCAB manifested better statistical features in analyzing the correlation of plasma RANTES level and AS conditions, having the highest correlation coefficient (0.786 for CCAB vs. 0.649 for cerebral AS burden, 0.520 for cardiac AS burden, 0.389 for cerebral AS severity, 0.460 for cardiac AS severity, 0.660 for cerebral AS extent, and 0.416 for cardiac AS extent). Moreover, our results implied that the integrated assessment of cardiac and cerebral AS conditions by CCAB was not merely applicable to patients with ICVD with serious AS in both cervicocephalic and coronary arteries ($r = 0.762$, $P < 0.001$). The significant correlation between plasma RANTES level and CCAB was also found in patients with ICVD who had AS ≥50% in just one of the cardiac and cerebral vessel beds ($r = 0.785$, $P < 0.001$), and the correlation coefficient was numerically larger. Therefore, even if patients with ICVD only had serious AS in single cardiac or cerebral vessel bed, it would be appropriate to evaluate the AS conditions of cervicocephalic and coronary

Table 3
Comparisons of plasma RANTES level and CCAB in patients with ICVD with and without multiple atherosclerotic stenosis ≥50%.

Characteristics	Plasma RANTES level (pg/mL)			CCAB (point)		
	Median (Q1, Q3)	Z value	P	Mean ± SD	t value	P
Presence of multiple stenosis ≥50% in cervicocephalic arteries (yes vs. no)	39979 (28268, 63786) vs. 25408 (19794, 31792)	−2.607	0.009[a]	23 ± 8 vs. 13 ± 6	−3.496	0.001[a]
Presence of multiple stenosis ≥50% in the coronary arteries (yes vs. no)	45890 (40177, 83861) vs. 30013 (23891, 43151)	−2.842	0.004[a]	28 ± 6 vs. 18 ± 8	−3.483	0.001[a]
Presence of stenosis ≥50% in both cervicocephalic and coronary arteries (yes vs. no)	44565 (29960, 82433) vs. 30066 (22342, 41028)	−2.478	0.013[a]	23 ± 9 vs. 18 ± 7	−2.244	0.031[a]

ICVD: ischemic cerebrovascular disease; CCAB: cardiocerebral atherosclerosis burden; RANTES: regulated upon activation, normal T-cell expressed, and secreted; SD: standard derivation.

[a] A P-value <0.05 was considered statistically significant.

arteries as a whole using CCAB and then analyze its relationship with plasma RANTES level.

Previously, AS conditions were often dichotomously evaluated by indices, such as the presence of AS, presence of stenosis ≥50%, and presence of multisegment stenosis ≥50%.[11,12,20] However, in this study, the plasma RANTES level was not obviously different in patients with ICVD with and without AS in cervicocephalic arteries, with and without stenosis ≥50% in cervicocephalic arteries, with and without AS in coronary arteries, and with and without stenosis ≥50% in coronary arteries (P > 0.05). In contrast, the plasma RANTES level had a significant and positive correlation with CCAB, and the correlation coefficient was as high as 0.786. Therefore, AS assessment with only a few dichotomous indicators might be

Table 4
General features of patients with ICVD with the highest and lowest tertiles of plasma RANTES level.

Characteristics	Total (n = 40)	Highest tertile (n = 13)	Lowest tertile (n = 13)	Statistical values	P
Age (year)	62 ± 8	66 ± 6	61 ± 9	−1.551[a]	0.134
Male	33 (82.5)	11 (84.6)	10 (76.9)	0.249[b]	0.618
Hypertension history	31 (77.5)	13 (100.0)	9 (69.2)	6.277[b]	0.012[d]
Diabetes mellitus history	18 (45.0)	3 (23.1)	6 (46.2)	1.552[b]	0.213
Hyperlipidemia history	33 (82.5)	11 (84.6)	12 (92.3)	0.383[b]	0.536
Coronary artery disease history	18 (45.0)	6 (46.2)	4 (30.8)	0.653[b]	0.419
Current smoking	14 (35.0)	6 (46.2)	4 (30.8)	0.653[b]	0.419
Overweight	22 (55.0)	9 (69.2)	6 (46.2)	1.418[b]	0.234
Onset of ICVD within 14 days	20 (50.0)	6 (46.2)	8 (61.5)	0.619[b]	0.431
Dual antiplatelet therapy	21 (52.5)	8 (61.5)	5 (38.5)	1.385[b]	0.239
SBP at admission (mmHg)	144.1 ± 24.2	137.1 ± 19.8	151.0 ± 20.8	1.752[a]	0.093
DBP at admission (mmHg)	83.6 ± 14.9	80.2 ± 15.9	86.9 ± 14.2	1.130[a]	0.270
Leukocyte count (10^9/L)	6.97 ± 2.18	6.46 ± 1.69	6.41 ± 1.74	−0.083[a]	0.935
Platelet count (10^9/L)	207 (176, 227)	197 (169, 224)	196 (165, 226)	−0.077[a]	0.939
Fasting glucose (mg/dL)	5.12 (4.56, 6.52)	4.91 (4.69, 6.15)	4.62 (4.36, 7.29)	−0.872[c]	0.383
LDL-C (mmol/L)	1.87 (1.62, 2.37)	1.76 (1.50, 2.17)	2.11 (1.84, 2.45)	−1.488[c]	0.137
Fibrinogen (g/L)	3.43 ± 0.76	3.18 ± 0.44	3.33 ± 0.81	0.591[a]	0.560
D-dimer (mg/L)	0.28 (0.17, 0.45)	0.26 (0.20, 0.38)	0.24 (0.14, 0.34)	−1.077[c]	0.281
hsCRP (mg/L)	1.15 (0.42, 2.79)	1.10 (0.49, 1.73)	0.56 (0.32, 3.00)	−0.579[c]	0.563

Data were shown as mean ± SD, median (Q1, Q3), or n (%).
ICVD: ischemic cerebrovascular disease; RANTES: regulated upon activation, normal T-cell expressed, and secreted; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-C: low-density lipoprotein cholesterol; hsCRP: hypersensitive C-reactive protein.

[a] t value.
[b] Chi-square value.
[c] Z value.
[d] A P-value <0.05 was considered statistically significant.

insufficient to fully reveal the relationship between plasma RANTES level and AS conditions in ICVD, while CCAB as a continuous variable would provide more quantitative and reliable estimation of the associations.

This study collectively evaluated AS conditions of cardiac and cerebral vessel beds in patients with ICVD and quantified them as a continuous variable CCAB and then analyzed its relationship with plasma RANTES level. The results demonstrated that the plasma RANTES level might be a promising inflammatory indicator for the overall AS burden of cervicocephalic and coronary arteries in ICVD. The method of assessing AS conditions by CCAB developed in this study might prompt future studies on AS-related circulating biomarkers and might improve understandings of the systematic initiation and progression of AS. However, this study was a single-center cross-sectional study with small sample size, and the results needed to be further confirmed. Especially as the circulating RANTES level would vary with races,[10] cautions must be taken in the generalization of our findings to other populations. Besides, CCAB was calculated based on the AS severity of each segment in cervicocephalic and coronary arteries; thus, the correlation of plasma RANTES levels and components of atherosclerotic plaques could not be evaluated in this study. In the future, more studies would be needed to determine whether plasma RANTES level and CCAB could be helpful in predicting the prognosis of ICVD. Moreover, a positive history of hypertension was the only general characteristic related to increased plasma RANTES level in this study. Other sensitive and specific factors associated with plasma RANTES level should be explored, which might aid in deciphering the underlying mechanisms of plasma RANTES level elevation in ICVD and its effects on AS development. With all these efforts, new targets for effective prevention and treatment of AS and ICVD might be found, and the prognosis of patients with cardiovascular and cerebrovascular diseases might be further improved.

In conclusion, increased plasma RANTES level was a significant related factor to the increase in CCAB in patients with ICVD. Plasma RANTES level had independent value to indicate the integrated AS burden of cervicocephalic and coronary arteries in ICVD. Evaluation of AS with CCAB as a continuous variable might better reflect the systematic relationship between circulating RANTES and AS in patients with ICVD compared with assessing AS conditions of a single cardiac or cerebral vessel bed.

Funding

Conflict of interest

None.

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Edited by Yi Cui