INVESTIGATING THE UNDERLYING METABOLIC MECHANISMS AND HEMODYNAMIC EFFECTS OF INTRACRANIAL ARTERY CALCIFICATION AS AN IMAGING BIOMARKER OF STROKE-SERIAL HOSPITAL-BASED CLINICAL RESEARCH

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Investigating the underlying metabolic mechanisms and hemodynamic effects of intracranial artery calcification as an imaging biomarker of stroke- Serial Hospital-based Clinical Research

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Declaration of Originality

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_____(Sign)

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Acknowledgements

As I wrap up my thesis, the song lyric "time to say goodbye" comes to mind. The end of my student life at the Hong Kong Polytechnic University is imminent. Reflecting on my PhD journey, I am overwhelmed with a myriad of emotions. I have met many people, experienced numerous events, all of which will remain as invaluable treasures and beautiful memories that will propel me forward.

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Publications and Presentations

Publication

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2. **Li X**, Chen J, Du H, Hua J, Zhang Y, Li X, Chen X. Association between Obstructive Sleep Apnea and Intracranial Artery Calcification Stratified by Gender and Body Mass Index: A Hospital-Based Observational Study. Neuroepidemiology. 2023;57(6):391-399.

3. **Li X**, Du H, Li X, Gao Q, Chen J, Chen X. Brachial-ankle pulse wave velocity is associated with intracranial artery calcification in acute stroke patients. Clin Neurol Neurosurg. 2023 Aug 2; 233:107918.

4. **Li X**, Du H, Li J, Li X, Gao Q, Chen X. Cerebral Arterial Stiffness as Measured Based on the Pulse Wave Velocity is Associated With Intracranial Artery Calcification in Patients With Acute Stroke. J Clin Neurol. 2023 Jul;19(4):338-343.

5. **Li X**, Du H, Li J, Chen X. Intracranial artery calcification as an independent predictor of ischemic stroke: a systematic review and a meta-analysis. BMC Neurol. 2023 Jan 16;23(1):21.

6. **Li X**, Du H, Yang W, Chen J, Li X, Chen X. The association of renal impairment with different patterns of intracranial arterial calcification: Intimal and medial calcification. Atherosclerosis. 2022 Dec; 363:42-47.

7. Du H, Zheng J, **Li X**, Dong Y, Cheng Y, Liu C, Hu J, Chen X. The correlation between

medial pattern of intracranial arterial calcification and white matter hyperintensities. Atherosclerosis. 2023 Sep;381:117247.

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Abstract Presentation

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Abstract

Background and purpose

Intracranial arterial calcification (IAC), a highly prevalent finding on head computed tomography (CT) scans, has been reported to be associated with ischemic stroke and cognitive impairment, previously regarded as a proxy indicator of intracranial atherosclerosis. However, the underlying pathophysiologic mechanism linking IAC and cerebral vascular diseases is yet unclear. It is thought that hemodynamics could significantly contribute to the development and progression of atherosclerotic plaques.

Increasing evidence from histopathological findings, coupled with advanced imaging techniques, has shown that IAC can be categorized into two distinct entities: intimal IAC, which often coincides with progressive atherosclerosis, and medial IAC, which is linked to artery stiffness. Furthermore, the difference between intimal and medial calcification in certain specific diseases may indicate the risk factors and processes that drive their development. In this thesis, our goal is to investigate the fundamental metabolic processes indicated by kidney dysfunction and the hemodynamic impacts of IAC, as revealed by pulse wave velocity in cerebral and peripheral arteries detected through ultrasound. Our research findings will provide valuable insights into the underlying mechanism accounting for two distinctive IAC patterns.

Methods and Materials

This series of hospital-based research consists of two main cohorts. Each cohort included consecutive patients who were admitted to the Department of Neurology and underwent thin-slice brain CT scans during their hospitalization. The extent of IAC, measured by IAC scores, was evaluated on non-contrast head CT images. The IAC was then classified as either intimal or medial calcification. Arterial stiffness was using Brachial-ankle pulse wave velocity

(baPWV) and carotid–cerebral pulse wave velocity (ccPWV). The function of kidney was assessed using the estimated glomerular filtration rate (eGFR), which was calculated based on a modified equation for estimating the glomerular filtration rate.

Results

In the first hospital-based cohort including a total of 516 patients (mean+/-SD) exploring the underlying metabolic mechanisms of two distinct IAC patterns, IAC was identified on brain CT in 440 patients (85.27%). Among these, 189 (42.95%) patients had predominant intimal calcifications, while 251 (57.05%) had predominant medial calcifications. Multivariate analysis revealed that lower eGFR level (eGFR <60 ml/min/1.73 m^2) was associated with higher IAC scores (OR 2.01; 95% CI, 1.50–2.71; $p < 0.001$). Medial calcification was more frequent in the group with a lower eGFR (eGFR <60 ml/ min/1.73 m^2) compared to the other two groups with eGFR 60 to 89 and eGFR >90 ml/min/1.73 m^2 (78.72% vs. 53.65%, $p < 0.001$; 78.72% vs. 47.78%, $p < 0.001$). In a multivariable analysis, impaired kidney function was associated with an increased likelihood of medial calcification presence in patients with eGFR <60 ml/min/1.73 m^2 (OR, 1.47; 95% CI, 1.05 to 2.06).

Simultaneously, the relationship between IAC and serum phosphorus concentration (SPC) was analyzed based on the same cohort. Data analysis showed that higher serum phosphorus was a significant risk factor for moderate/severe IAC in patients with eGFR ≥60 ml/min/1.73 m^2 (OR, 1.27; 95% CI, 1.01-1.59; P <0.05) and eGFR <60 ml/min/1.73 m^2 (OR, 1.92; 95% CI, 1.04-3.57; P <0.05), using those with mild IAC as the reference group. However, a higher SPC was linked to an increased likelihood of medial calcification only in patients with eGFR <60 ml/min/1.73 m^2 (OR, 1.67; 95% CI, 1.08 to 2.61).

In the second hospital-based cohort including 143 stroke patients (18 to 80 years old), investigating the relationship between IAC and baPWV, a higher prevalence of IAC was noted

across increasing baPWV quartiles (Q1: 53 %, Q2: 69 %, Q3: 86 %, Q4: 94 %, P < 0.001). IAC scores were also accelerated with increasing ccPWV values (1.60 ± 1.71; 2.56 ± 1.99; 3.44 ± 1.91; 4.64 ± 1.58. P < 0.001). After adjusting age and hypertension, the odds ratio (95 % confidence interval) for the IAC scores was 1.61 (1.06–2.45; P = 0.025) in the top quartile of baPWV compared with those in the lowest quartile.

Meanwhile, the same cohort was used to examine the relationship between IAC and ccPWV. The prevalence of IAC also increased in line with the ccPWV quartile, with rates of 54%, 76%, 83%, and 89% for quartiles 1, 2, 3, and 4, respectively (p<0.001). IAC scores followed a similar pattern, with median [interquartile range] values of 0 [0–2], 3 [2–4], 4 [2–5], and 5 [4–6], respectively (p<0.001). After further adjusting for age and hypertension, a significant correlation was only observed between quartiles 3 and 4 of ccPWV and IAC scores. The odds ratio (95% confidence interval) for the IAC scores was 1.78 (1.28–2.50) (p=0.001) in quartile 4 of ccPWV and 1.45 (1.07–1.95) (p=0.015) in quartile 3 when compared with quartile 1.

Conclusions

Our research findings, based on two cohorts of hospitalized patients, addressed two clinical questions related to IAC. The findings from the first cohort indicated that impaired renal function was independently associated with a higher degree of calcification in intracranial arteries, particularly medial calcification. This highlights a distinction between two types of intracranial arterial calcification and suggests the potential for targeted prevention of lesion formation and cerebrovascular diseases. Our second cohort-based study found that the degree of cerebral arterial calcification was correlated with peripheral arterial stiffness, as evaluated by baPWV, and cerebral arterial stiffness, as measured by ccPWV, in patients with acute ischemic stroke. This suggests that IAC significantly changes the hemodynamic parameters

within cerebral arteries and could be a contributing factor to the onset of cerebral artery diseases.

Longitudinal studies will be performed to further validate the clinical significance of IAC in predicting cerebrovascular disease in the general population, and in predicting clinical outcome of stroke patients.

List of Abbreviations

AIS, acute ischemic stroke

ARI, autoregulation index

BA, basilar artery

baPWV, Brachial-ankle pulse wave velocity

BMI, body mass index

CAC, coronary artery calcification

CAD, coronary artery disease

CCP, critical closing pressure

ccPWV, carotid–cerebral pulse wave velocity

CSVD, cerebral small vessel disease

CT, computed tomography

eGFR, estimated glomerular filtration rate

ESRD, end-stage renal disease

HbA1c, Hemoglobin A1c

HDL-C, high-density lipoprotein-cholesterol

ICA, internal carotid artery

ICAS, intracranial atherosclerotic stenosis

LRNC, lipid-rich necrotic core

LDL-C, Low-density lipoprotein cholesterol

MCA, middle cerebral artery

MRI, magnetic resonance imaging

PI, pulpability index

PTH, parathyroid hormone

SBP, systolic blood pressure

SPC, serum phosphorus concentration

TCD, Transcranial Doppler

TFA, function analysis

TG, Total Cholesterol

VA, vertebral artery

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Chapter 1. General Introduction

1.1 Background

1.1.1 Intracranial arterial calcification

Intracranial arterial calcification (IAC), which is considered an active process of atherosclerosis, was first observed by ex vivo radiography and microscopic pathology in the early 1960s.[1] According to our previous clinical studies, IAC was a highly prevalent finding on brain computed tomography (CT) scans among the general populations.[2] However, the clinical implications of IAC are also profound, as it is associated with an increased risk of cerebrovascular diseases. Several previous studies have demonstrated a strong association between IAC and stroke, particularly ischemic stroke.[3] Moreover, IAC has been linked to cognitive impairment and vascular dementia, suggesting a potential role in the pathogenesis of neurodegenerative diseases.[4] However, the underlying mechanisms between IAC and these diseases remains unclear.

Currently, predicting the progression of vascular calcification requires a multifaceted approach that incorporates various predictive factors and utilizes advanced imaging. Firstly, identifying and monitoring traditional cardiovascular risk factors such as age, hypertension, diabetes, dyslipidemia, smoking, and chronic kidney disease can provide insights into the likelihood of calcification progression. Secondly, research into biomarkers associated with vascular calcification may provide additional predictive value. Furthermore, machine learning algorithms can analyze large datasets from patient records, imaging, and biomarker profiles to identify patterns that predict calcification progression. Finally predictive models can be developed to estimate the

risk of progression based on individual patient data.

1.1.2 Different pattern of intracranial arterial calcification

Previous studies mostly evaluated IAC severity using a visual grading scale based on vessel wall circumference and calcified area thickness. This method, however, lacks differentiation of IAC patterns, potentially causing controversies. Recently, an advanced grading system was developed, classifying IAC patterns through cross-validation of imaging and histological features. This optimized IAC scoring system makes the quantification of calcification more detailed and is more conducive to exploring the formation mechanisms of different types of calcifications and its correlation with clinical consequences.

Depend on the location of the calcification within the vessel, vascular calcification was categorized into two different forms: intimal and medial calcifications, intimal calcification is a marker of atherosclerosis and is exclusively associated with arterial luminal stenosis, while medial calcification seems to relate to increased arterial stiffness and reduced arterial compliance.[5-11] Therefore, exploring the underlying mechanisms or risk factors associated with these two patterns of calcification is essential, as they each hold distinct clinical significance in the context of vascular calcification. Previous studies indicated that intimal calcifications have been associated to older age, male sex, hyperlipidemia, smoking, hypertension, and previous history of cardiac disease. Conversely, medial calcifications are more associated with diabetes and renal dysfunction.

In coronary artery disease studies, a different distribution of intimal calcification and medial calcification was found in case of renal impairment in both animal studies and clinical trials, suggesting the unique mechanism of renal impairment in their development.[12-16] However, to the best of our knowledge, there are no clinical studies to explore the relationship between kidney dysfunction and IAC patterns. More importantly, considering that calcification in different vascular layers may lead to different clinical outcomes, understanding the relationship between renal impairment and IAC patterns may help optimize cerebrovascular disease prevention and therapeutics.

1.2 Objectives and organization of the thesis

1.2.1 Objectives

This hospital-based study aimed to investigate the underlying metabolic mechanisms and hemodynamic effects of IAC. It consisted of four major objectives:

1) to investigate the association between kidney function and the two patterns of IAC, which could clarify the underlying mechanisms of intimal or medial calcification and its clinical consequence.
2) to determine whether the serum phosphorus concentrations (SPC) are associated with the degree and pattern of IAC in patients with normal renal function or mild-moderate renal impairment.
3) to examine the association of brachial-ankle pulse wave velocity(baPWV) with the presence and degree of IAC in patients with acute ischemic stroke.

4) to determine the association of IAC with arterial stiffness as reflected by the pulse wave velocity between the carotid and middle cerebral arteries using transcranial Doppler sonography in patients with acute stroke.

1.2.2 The organization of the thesis

This study is divided into five chapters. Chapter 1 provides the general introduction of this thesis that summarized the background and the objectives of the study. Chapter 2 is literature review, the association between IAC and the risk of ischemic stroke occurrence or poor prognosis was assessed by using a meta-analysis of available studies. The correlation between kidney dysfunction and intimal or medial calcification is described in Chapter 3-1, which provide useful information to explain the underlying mechanism accounting for two different patterns of IAC. In Chapter 3-2, we explored the association of SPC with the degree and pattern of IAC in patients with normal renal function or mild-moderate renal impairment, aiming to determine whether imbalance in serum calcium and phosphorus levels due to impaired renal function is one of the main potential mechanisms leading to the formation of medial calcifications. Chapter 4-1 focuses on the correlation between IAC and peripheral arterial stiffness as measured by baPWV. In Chapter 4-2, based on our previous method that used a novel original time and distance assessment technique to directly evaluate the human carotid-cerebral PWV (ccPWV), we aimed to determine the effect of IAC on cerebral artery stiffness among patients with stroke. Chapter 5 is conclusions of this thesis and perspectives for future studies.

Chapter 2. Literature Review:

Intracranial artery calcification as an independent predictor of ischemic stroke: a systematic review and a meta-analysis

2.1 Introduction

Stroke has remained in the top three causes of death and a major cause of disability globally, and its absolute number of cases increased substantially from 1990 to 2019.[17] According to a population-based screening project in China, the prevalence of stroke in China and most provinces has continued to increase in the past 7 years (2013−2019).[18,19] It has been shown that major arteries' intracranial atherosclerotic stenosis (ICAS) represents a common cause of ischemic stroke worldwide. Intracranial arterial calcification (IAC), which is considered an active process of atherosclerosis, was first observed by ex vivo radiography and microscopic pathology in the early 1960s. According to our previous clinical studies, IAC was a highly prevalent finding on brain computed tomography (CT) scans among the general populations and patients with stroke or transient ischemic attack.

IAC is known to be an essential risk factor for cerebral infarction and an essential marker of ICAS.[20-24] Several recent studies have shown a significant association between IAC and first-ever ischemic stroke risk. Moreover, the value of IAC as a predictor of recurrent stroke risk was also demonstrated in these studies. Assessing IAC by using quantitative Agatston score, our recent study found that IAC was a strong risk factor for recurrent stroke and post-stroke mortality.[22,25-27] However, it is important to note that the association between calcification and stroke has not gone undisputed, and these findings have never been pooled before. To provide clarity, we aim to inform the relationships between IAC and future ischemic stroke or mortality in the form of a

meta-analysis of available studies.

2.2 Methods

This study was preformed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. It was registered in PROSPERO international prospective register of systematic reviews (CRD42021281357).

2.2.1 Data sources and search strategy

We searched Medline, Cochrane, Web of Science, Google Scholar databases from inception to June 30, 2022. Prospective or retrospective cohort and case-control studies were included. Relevant keywords, phrases and medical subject headings (MeSH) terms were used. the following search keywords in different combination were used: stroke, intracranial artery, calcification or calcified or calcium. Furthermore, we hand-searched reference lists of included studies to find additional studies. Only English language studies and accompanied by full-length peer reviewed papers were included. Two reviewers independently screened and performed parallel assessments of the manuscripts. The following search strategy was used for Web of Science and modified to suit other databases:

#1 stroke.

#2 intracranial artery.

#3 calcification OR calcified OR calcium.

#4 #1 AND #2 AND #3.

We also used the “cited by” function of Google Scholar to minimize the risk of missing

data.

2.2.2 Study selection

Studies screened and examined by titles and abstracts after removing over-lapping and duplicate articles. Only studies that met the following criteria were included in this meta-analysis: limited to human subjects; report risk ratio (RR) or odds ratios (OR) and their corresponding 95% confidence intervals (CI) of stroke relating to IAC, and at least one of the following outcomes should be reported: occurrence, recurrence or mortality. Letters, comments, editorials, case reports, proceedings, and personal communications were excluded.

2.2.3 Data extraction

Data extraction was independently performed by two reviewers using a standard form. the data extracted included the following information: first author, study design, publication year, country of origin, sample population demographic characteristics, gender, sample size, endpoint, and covariates adjustment in each study.

2.2.4 Quality assessment

The quality assessment for observational studies included in this meta-analysis were assessed using the Newcastle Ottawa Scale (NOS). This scale comprised of three domains (selection, comparability, and outcome) with a maximum score of 9 points. Tus, the risk of bias categorized into three groups: high (0-3), moderate (4-6), and low (7-9).

2.2.4 Statistical analysis

Meta-analysis was performed using either fixed-effects models (I^2<50.0%) or, in the

presence of heterogeneity (I^2>50.0%), random-effects models. Heterogeneity across studies was assessed by using both the Q test and the I^2 statistic (ranging from 0% for perfect homogeneity to 100% for extreme heterogeneity). An I^2 value >50% indicates significant heterogeneity. We also performed a subgroup analysis to further assess the association between IAC and the risk of stroke events according to clinical characteristics, sample size and study design. Pooled effects were calculated, and a two-sided P value <0.05 was generally considered to indicate statistical significance. Publication bias was not performed as enough studies are necessary for this type of analysis. the data analysis was done using Stata 16.0 software.

2.3 Results

2.3.1 Data sources and searches

Our initial electronic and hand search of all the databases identified 1042 records. After initial screening of the titles and abstracts, we reviewed the full text of the remaining 63 articles and rejected 29 citations that did not report the effect sizes or full detailed data, 19 were excluded because the clinical outcome was not stroke and 3 further studies because they used the same data source. Overall, a total of 12 studies involving 9346 participants were eligible for quantitative synthesis (meta-analysis).[22,28-37] The flow chart for study inclusion is shown in **Figure 2-1**.

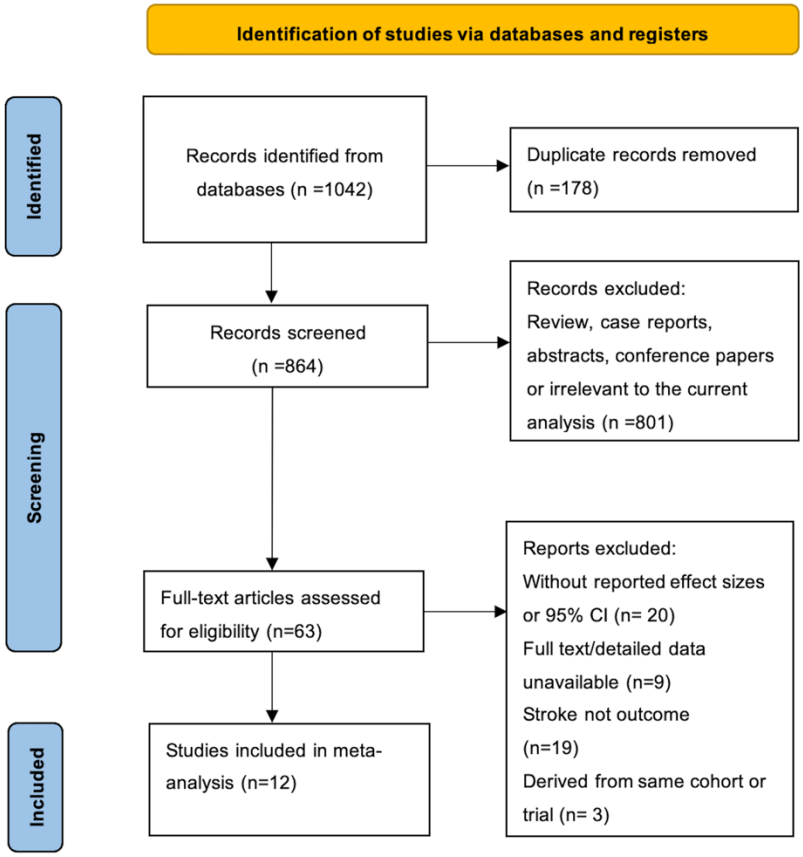

Figure 2-1 Flow chart for study screening and selection

2.3.2 Study characteristics of included studies

The main characteristics of the included studies were presented in Table 1. Publication dates ranged from 2003 to 2020; the sample sizes of included studies ranged from 99 to 2323 participants; the average age of subjects was 67.3years (range 51.0–73.6years). Among these 12 studies, eight reported the risk of stroke occurrence, four reported the association between IAC and post-stroke mortality, only 3 reported the risk of stroke recurrence, two were from the United States, six were from Europe, and four were from Asia. The quality assessment of included study is presented in **Table 2-1**. The risk of

bias for all 12 studies included in the meta-analysis was moderate or above with high quality.

Table 2-1 Characteristics of included studies.

Trial	Region	Cases	Trial design	Age (years)	Male (%)	Outcome variables	aOR (95% CI)	Risk of bias
Douglas 2003	USA	322	Cohort study	73	53	OC	0.96 (0.1-8.97)	Moderate
Chen 2007	CHINA	357	Case-control study	65.9	54.1	OC	3.17 (1.25-8.04)	Moderate
Bugnicourt 2009	France	511	Case-control study	65.7	56.8	OC	1.89 (1.13-3.14)	Moderate
POWER 2011	UK	529	Cohort study	59	61	OC & MO	OC: 1.48 (1-2.23) MO: 2.17 (1.22–3.87)	Low
Koton 2012	Israel	1049	Cohort study	70	59	MO	Mild: 1.6 (0.6–4.3) Severe: 1.0 (0.4–3.0)	Moderate
Lee 2014	Korea	1017	Cohort study	67.7	56.7	RE & MO	Mild: RE: 1.49 (.83-2.67) MO: 0.51 (.19-1.37) Severe: RE: 2.00 (1.07-3.71)	Moderate

							MO: 0.54 (.19-1.53)	
Bos2014	Europe	2323	Cohort study	69.5	47.8	OC	1.39 (0.98-1.99)	Moderate
Kao 2015	Netherlands	1872	Case-control study	NA	53.7	OC	1.02 (0.98, 1.07)	Low
Quiney 2017	USA	99	Case-control study	55	58.6	OC	2.2（1.2-3.9）	Moderate
Zhang 2019	China	125	Case-control study	NA	NA	OC	1.98 (1.45~2.69)	Low
Magdič 2020	Slovenia	448	Case-control study	76	47.3	RE & MO	RE: 3.13 (1.35-7.20) MO: 1.22 (0.93-1.56)	Moderate
Wu 2020	China	694	Cohort study	71.6	50.3	RE & MO	RE: 1.23 (0.57, 2.66) MO: 3.17 (0.42-23.79)	Low

Abbreviations: USA, United States of America; NA, not available; OC, occurrence; RE, recurrence; MO, mortality; aOR, adjusted odds ratio; CI, confidence intervals.

2.3.3 Association between IAC and ischemic stroke incidence

Compared with those without IAC, among subjects with IAC had an elevated risk of ischemic stroke (OR 1.62; 95% CI 1.18–2.23, p=0.003; **Figure 2-2**). A random-effects model was used to assess the pooled outcome due to the included studies' extreme heterogeneity (I^2=82.3%, P< 0.001). Considering the relatively high heterogeneity, a sensitivity analysis following the leave-one-out approach was performed to show that Kao et al. study had the most significant influence on the heterogeneity, and the pooled

RR without this study was 1.75 (95% CI, 1.47–2.08) with the I^2 value reduced to 0%. No association between the modifiers age, study design, sample size, country, study quality and IAC were identified in meta-regression analyses (p=0.46, p=0.32, p=0.25, p=0.13, and p=0.20, respectively).

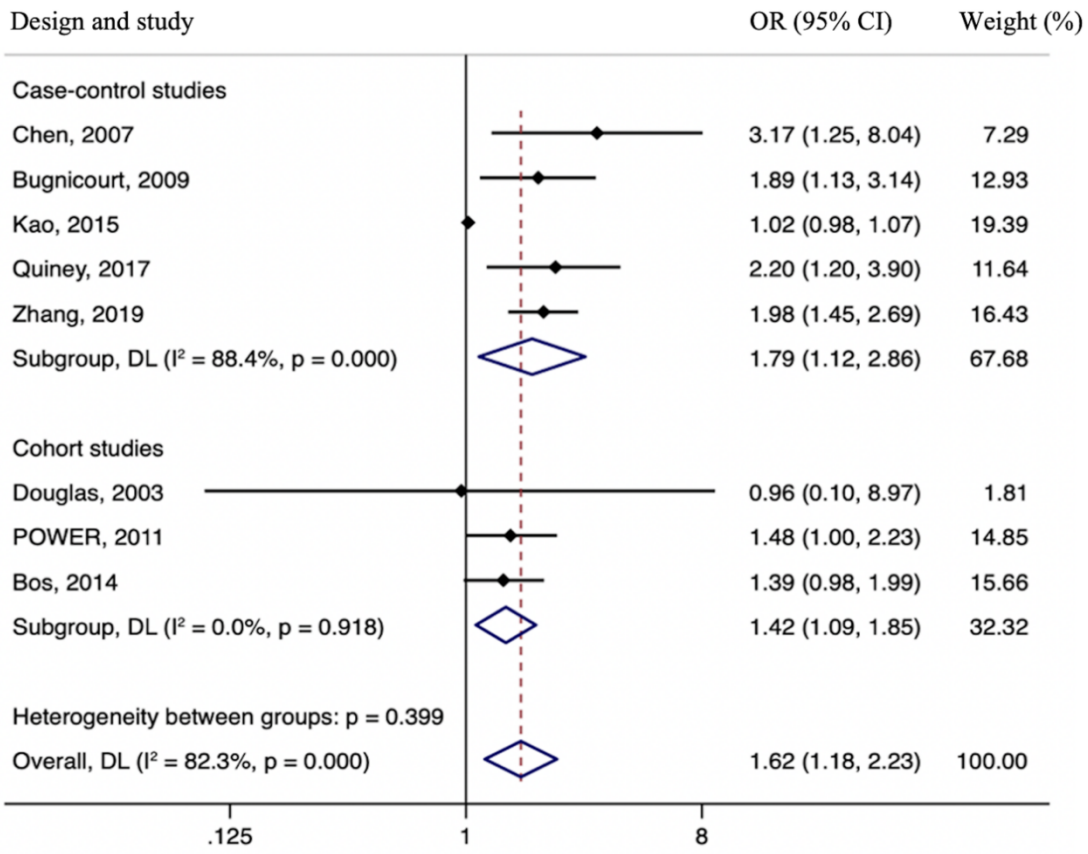

Figure 2-2 Meta-analyses of hazard ratios for the association between intracranial arterial calcification and stroke incidence

2.3.4 Association between IAC and ischemic stroke recurrence

Results from three studies demonstrated that the presence of IAC related to a higher risk of stroke recurrence (OR 1.77; 95% CI, 1.25–2.51, p=0.001; **Fig 2-3**) without significant heterogeneity (P=0.373, I^2 =3.9%).

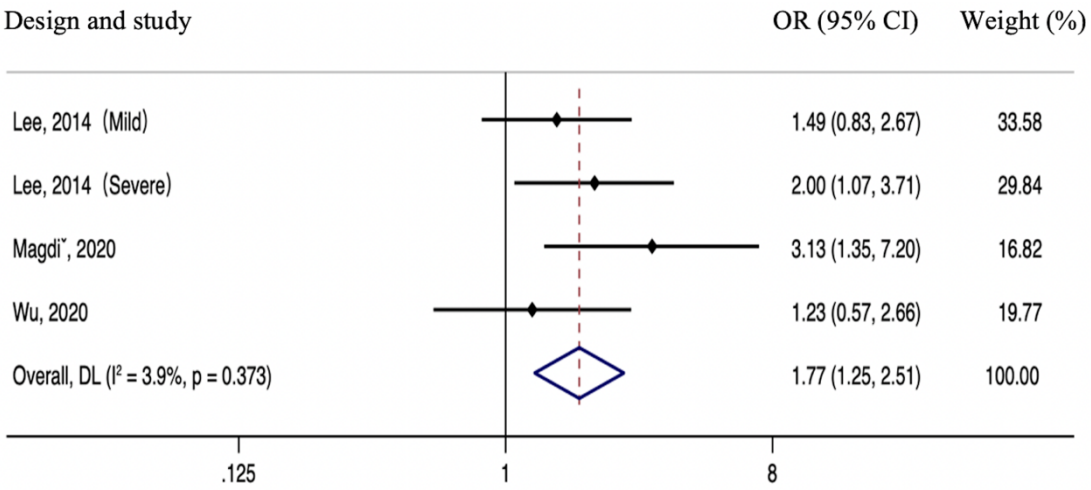

Figure 2-3 Meta-analyses of hazard ratios for the association between intracranial arterial calcification and stroke recurrence

2.3.5 Association between IAC and post-stroke mortality

Five studies reported post-stroke mortality rates. Pooled results of the meta-analysis showed a lack of correlation between IAC and post-stroke mortality (pooled OR 1.12; 95% CI 0.80–1.56, P=0.504) without significant heterogeneity among the studies (P=0.081, I^2=44.6%; **Fig 2-4**).

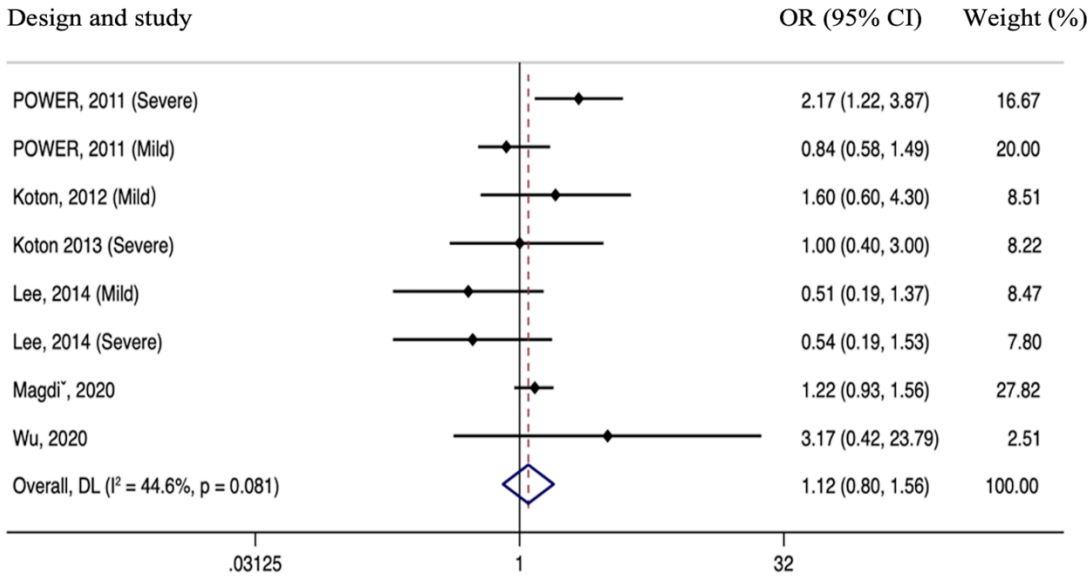

Figure 2-4 Meta-analyses of hazard ratios for the association between intracranial arterial calcification and stroke mortality

2.3.6 The results of subgroup analysis between IAC and ischemic stroke

In the study design subtype, a statistically significant effect of IAC on first stroke risk was observed in both cohort studies (OR 1.42; 95% CI 1.09–1.85, p< 0.001) and case-control studies (OR 1.79; 95% CI 1.12–2.863, p< 0.001). When stratified by continent, IAC was positively related with increased risk among studies performed in European (OR, 1.28; 95% CI 0.93–1.64, p< 0.001), North American (OR, 2.09; 95%CI 0.80–3.69, p=0.001) and Asian (OR, 2.02; 95% CI 1.41–2.63, p< 0.001). In the further analysis by the number of cases, studies that included a small sample, that is, <500 patients (OR 2.08; 95% CI 1.60–2.70, p< 0.01) had a higher risk of ischemic stroke than those studies with more than 500 cases (OR 1.33; 95% CI 0.99–1.79, p=0.06). The results of subgroup analyses are shown in **Table 2-2**.

Table 2-2 Summary risk estimates of the subgroup analysis results of ICAC and stroke occurrence.

Subgroup	Studies (n)	OR (95%CI)	I^2 (%)	P value
Total	8	1.62 (1.18, 2.23)	82.3	<0.001
Design				
Case-control study	5	1.79 (1.12, 2.86)	88.4	<0.001
Cohort study	3	1.42 (1.09, 1.85)	0	<0.001
Location				

Asia	2	2.02 (1.41, 2.63)	0	<0.001
Europe	4	1.28 (0.93, 1.64)	0	<0.001
North America	2	2.09 (0.80, 3.69)	57.1	0.001
Risk of bias				
Low	3	1.41 (0.89, 2.25)	90	0.14
Moderate	5	1.66 (0.90, 3.08)	2.8	<0.001
No. of cases				
< 500	4	2.08 (1.60, 2.70)	0	<0.001
> 500	4	1.33 (0.99, 1.79)	73.9	0.06

Abbreviations: OR, odds ratio; CI, confidence intervals.

2.3.7 Publication bias

Visual inspection and Egger's test indicated an asymmetric funnel plot (p=0.007). As only eight studies assessing the risk of stroke occurrence were included in the present meta-analysis, we could not entirely rule out the presence of publication bias.

2.4 Discussion

In this meta-analysis of studies on IAC and ischemic stroke, a positive correlation between IAC and stroke occurrence was found in the analysis (OR 1.62; 95% CI 1.18–2.23) and between IAC and stroke recurrence was also found (OR 1.77; 95% CI, 1.25–2.51). However, there was no significant correlation between IAC and post-stroke mortality.

IAC is a common incidental finding on brain CT scan in the general population and, although most of calcifications are viewed as innocent without any prognostic significance, others are related to adverse clinical outcomes.[38-40] Several studies showed that IAC was linked with an increased risk of stroke independently of risk factors for heart disease and other cardiovascular disease.[2,41,42] Although many diverse hypotheses have been proposed in previous studies, the mechanisms related IAC to ischemic stroke have not, as yet, been fully understood. First, vulnerable/unstable plaque with a large lipid-rich necrotic core (LRNC), thinning of the fibrous cap (FC), inflammation, and intraplaque hemorrhage (IPH), is more likely to rupture and may cause a brain ischemic event.[43,44] Second, highly calcified arteries may, in some cases, have a severe narrowing or occlusion of the lumen leading to hemodynamic disturbances; also, the presence of large volumes of calcification reflects large plaques, which may be an essential source of embolic to the brain.[45-47] IAC may result in impaired arterial endothelial function, leading to damaged cerebral blood flow autoregulation and cerebrovascular reactivity, which might be a potential mechanism accounting for the lacunes and stroke.[48-50] In the current subgroup analysis stratified by region, IAC was positively related with increased risk among studies performed in European, North American and Asian, suggesting that IAC may be one of the most important risk factors for ischemic stroke worldwide. When stratified by study sample size, studies with small sample sizes seem to produce larger effect sizes than large studies.

Although this study showed that IAC was closely related to the recurrence of ischemic stroke, it must be note that there are still few studies in this fields and the conclusions are inconsistent. In the term of IAC impact on recurrence after ischemic stroke, Wu et al. showed a higher degree of IAC was associated with a high risk of stroke recurrence in patients with cerebral small-vessel disease, which might indicate chronic calcification observed in large intracranial arteries may have potential impacts on the cerebral vascular bed extending to small blood vessels.[51] Moreover, the predictive value for IAC for poor outcomes has not been established. Magdiˇ et al. reported that the presence of vertebrobasilar artery calcification was significantly related with the overall risk of long-term death and other cardiovascular events after ischemic stroke.[33] However, Koton et al. found that IAC was not significantly associated with mortality and poor functional outcome in patients with acute ischemic stroke after adjusting for traditional risk factors.[35]

In our meta-analysis, no significant correlation was found between IAC and post-stroke mortality, still, the investigation of the link between IAC and stroke prognosis may have important clinical significance.[52,53] Although there is still controversy regarding the relationship between calcification and instability of plaque, the amount and extent of multiple intracranial arterial calcifications can reflect the degree of evolution of atherosclerosis and the possibility of the presence of unstable plaques.[54-57] Furthermore, previous study showed that subjects with grade 3 or 4 calcification of an intracranial artery on brain CT were more likely to have a significant stenosis (greater than 50%)

on cerebral angiography.[58,59] At this point, IAC would be a useful marker for future cerebrovascular events after ischemic stroke, especially in non-cardioembolic infarction. Further evaluation of the potential causal link between calcifications in the intracranial vessels and poor outcomes after ischemic stroke is vital for future research to develop prevention strategies and implications.

To our best of our knowledge, this is the first systematic review and meta-analysis, focus on the relationships between IAC and ischemic stroke. As a common and easily identifiable finding on brain CT, IAC may be a useful indicator to predict the risk and outcome of ischemic stroke. Several limitations of this study should be considered. First, the studies included in this meta-analysis were either cohort studies or case-control studies, thus, the causality between IAC and the risk of ischemic stroke remains unclear. Second, the number of articles included seems too small, therefore, a larger-sized, prospective study is warranted to further investigate the association between arterial calcification and stroke prognosis. Third, we excluded articles that were not published in English to ensure the quality of studies, but it might lead to publication bias. Fourth, it would be very beneficial if subgroup analysis could be conducted based on the arteries involved. Although some recent studies aimed to explore the relationship between different patterns of intracranial arterial calcification and atherosclerotic disease, the exact patterns of involvement in the intracranial arteries and stroke were not reported in the included studies.[60] Finally, different patterns of IAC may lead to

different clinical outcomes, but all studies included in this meta-analysis did not report the relationship between IAC patterns and ischemic stroke.

2.5 Conclusions

In conclusion, the findings from this study indicate an association between IAC and increased stroke risk. Further carefully designed and well-conducted studies with prospective design are needed to conduct to identify our results.

Chapter 3:

The Underlying Metabolic Mechanisms of Intracranial Artery Calcification

Chapter 3-1:

The Association of Renal Impairment with Different Patterns of Intracranial Arterial Calcification: Intimal and Medial Calcification

3.1.1 Introduction

Intracranial arterial calcification (IAC) can be easily identified on computed tomography (CT).[38] The highest prevalence of IAC was seen in the internal carotid artery (80.4%), followed by the vertebral artery (35.6%), basilar artery (7.3%) and middle cerebral artery (4.5%).[34] Previously regarded as a proxy indicator of intracranial atherosclerosis (ICAS), IAC has been reported to be a risk factor of ischemic stroke, white-matter disease or microbleeds, and cognitive impairment.[61,62] Our histological study based on 32 adult autopsy cases classified IAC as intimal or medial calcification according to its specific location in the vessel wall and demonstrated that intimal calcification was more closely associated with ICAS in our subsequent multimodal imaging-based comparison study.[55,60,63] Moreover, differences between intimal and medial calcification in some specific diseases may reflect risk factors and mechanisms behind their development.

Kidney dysfunction affects 13% of the population worldwide and up to 35% of individuals aged 70 years or older.[61,64,65] Kidney dysfunction is correlated with coronary artery calcification (CAC) but the association of impaired kidney function with IAC is less studied.[12] In coronary artery disease studies, a different distribution of intimal calcification and medial calcification was found in case of renal impairment in both animal studies and clinical trials, suggesting the unique mechanism of renal impairment in their development.[13,16,66] However, to the best of our knowledge, there are no clinical studies to explore the relationship between kidney dysfunction and IAC types. More

importantly, considering that calcification in different vascular layers may lead to different clinical outcomes, understanding the relationship between renal impairment and IAC patterns may help optimize cerebrovascular disease prevention and therapeutics.

In this hospital-based study, we aimed to investigate the correlation between kidney dysfunction and intimal or medial calcification, which will provide useful information to explain the underlying mechanism accounting for two different patterns of IAC.

3.1.2 Methods

3.1.2.1 Study participants

This study included consecutive hospitalized patients who underwent brain CT scans and were admitted to the Department of Neurology, The Second Affiliated Hospital of Guangzhou Medical University, between January 1, 2021, and March 1, 2022. Inclusion criteria were as follow: (1) age ≥18 years; (2) having performed brain CT with 0.625-mm slice thickness as measurement of calcification; and (3) estimating estimated glomerular filtration rate (eGFR) using modified glomerular filtration rate estimating equation. The exclusion criteria were: (1) poor CT imaging quality; (2) insufficient clinical data for analysis.

This study was approved by the clinical ethics committees of the participating hospital. The following clinical information was obtained from the electronic medical records of

the patients: age; sex; diagnosis; total cholesterol [TC], low-density lipoprotein-cholesterol [LDL-C], high-density lipoprotein-cholesterol [HDL-C], triglyceride [TG], hemoglobin A1c [HbA1c], homocysteine, eGFR; past medical history; medication history. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic pressure ≥90 mmHg or history of hypertension. Diabetes mellitus (DM) was determined using a 75 g oral glucose tolerance test, or as HbA1c ≥ 6.0%, or medical history of diabetes.

3.1.2.2 Image acquisition

CT imaging was performed using a 64-row multidetector scanner without contrast administration. All CT exams (120 kVp, 170 mAs, 1-sec rotation time) were acquired in axial mode with tilting along the occipito-meatal line, covering the region from skull base to vertex. For detection of subtle or thin calcifications, only CT exams with 0.625-mm slice thickness were included.

3.1.2.3 IAC assessment

Reconstructed CT images were independently evaluated by two readers (JR. C. and XL. L.) with at least two years of experience in CT image interpretation, who were blinded to clinical information of the patients. The presence of IAC was assessed using visual grading method. Seven main intracranial arteries (bilateral internal carotid artery [ICA] C2–C7 segments, bilateral middle cerebral artery [MCA], bilateral vertebral artery [VA] V4 segments, and basilar artery [BA]) were assessed. The presence of IAC was defined as the hyperdense artery sign considered with a density of more than 130 Hounsfield

units. As previously described, the severity of IAC was evaluated by grading values (extent and thickness) for each cerebral artery, and a highest composite CT score of 0–2, 3–5 and 6–8 was classified as mild, moderate, and severe IAC, respectively. The vessel with the highest score was used for the final score (**Figure 3-1**).[67]

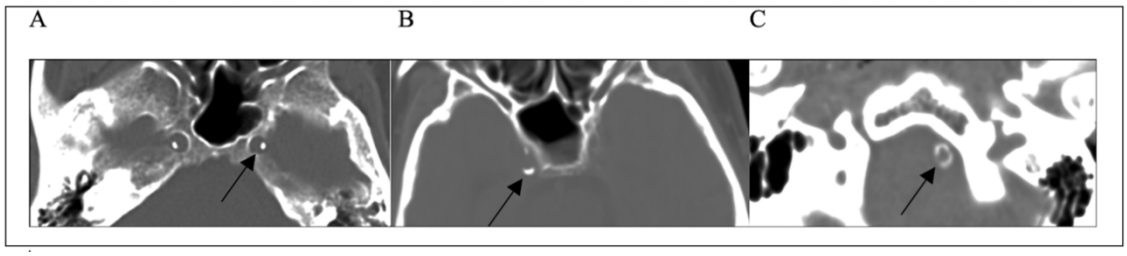

Figure 3-1 Examples of IAC scores on CT image. According to Babiarz's visual grading scales, IAC were graded as follows. (A) 1 point for extent and 1 point for thickness (mild). (B) 2 points for extent and 2 points for thickness (moderate). (C) 4 points for extent and 2 points for thickness (severe).

IAC patterns were classified according to a previous calcification scoring method. The morphological patterns of calcifications were scored as follows (**Figure 3-2**): calcification circularity: absent (0 point); dots (1 point); <90◦ (2 points); 90–270◦ (3 points); 270–360◦ (4 points). Calcification thickness: thick ≥1.5 mm (1 point); thin <1.5 mm (3 points). Calcification morphology: indistinguishable (0 point); irregular/patchy (1 point); continuous (4 points). The sum of the calcification scores was used to classify intimal (1–6 points) and medial calcifications (7–11 points).[5] For the patients with several calcifications, we counted the number of calcifications in each patient as well as comparing which pattern of calcification had a higher proportion. Thus, all patients

were divided into two groups: patients with predominant medial calcifications and patients with predominant intimal calcifications.

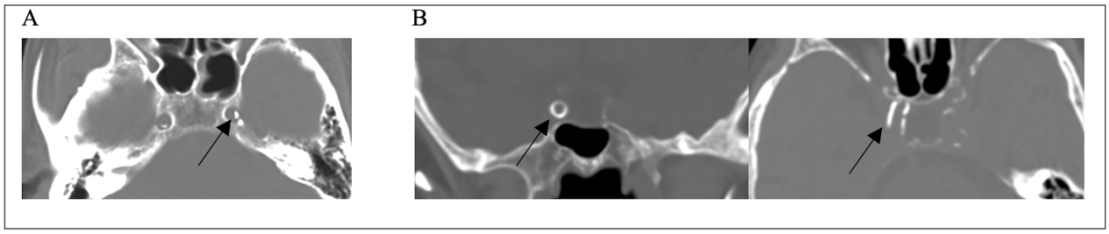

Figure 3-2 Examples of calcification score for categorizing ICA calcification patterns.

(A) 1 point for circularity, 3 points for thickness and 1 point for morphology (intimal calcification).

(B) 4 points for circularity, 3 points for thickness and 4 points for morphology (medial calcification).

3.1.2.4 Kidney function

Serum levels of creatinine were measured at admission. The eGFR was assessed by calculating serum creatinine (sCr) concentrations using the modified glomerular filtration rate estimating equation for Chinese subjects: eGFR (ml/min/1.73 m^2) = 175 × (sCr)¯1.234 × (age)¯0.179 (0.79 if female). eGFR >90 ml/min/1.73 m2 would indicate normal kidney function, eGFR 60–90 ml/min/1.73 m^2 mildly reduced kidney function and eGFR <60 ml/min/1.73 m2 as decreased eGFR.

3.1.2.5 Statistical analysis

Baseline demographics, clinical characteristics and laboratory findings were compared between the three eGFR groups. Continuous variables were presented as mean and standard deviation (SD), and categorical variables were presented as numbers and percentages. Characteristics of participants were compared according to the severity of

renal function using the chi-squared test for categorical data. Continuous variables were compared across groups using the Kruskal-Wallis test.

Logistic regression analysis was used to determine the association of eGFR with degree and patterns of IAC, adjusting for age and sex (model 1), body mass index (BMI), smoking status, alcohol use, SBP and DBP (model 2), and history of coronary artery disease (CAD), history of stroke, history of hypertension, history of diabetes, TG, TC, LDL, HDL, HbA1c, Hcy, and the use of antihypertensives medication, hypertension medication, antiplatelets medication and statins medication (model 3). A two-sided p value < 0.05 indicated statistical significance. Statistical analyses were performed using the SPSS software version 26 (IBM, Chicago, Illinois, USA).

3.1.3 Results

3.1.3.1 Participants

A total of 541 patients with thin-slice CT scans and renal function tests were initially included in this study. After excluding 20 patients with image artifacts on CT and 5 patients with insufficient clinical data, 516 patients were included.

Baseline characteristics of the participants are shown in **Table 3-1**. The mean age was 68.4 ± 11.1 years, and 269 (52.1%) patients were male. The mean value of eGFR was 83.24 ml/min/1.73 m^2 (SD, 25.34) and 101 (19.57%) had a value < 60 ml/min/1.73 m^2.

Compared with the normal kidney function group, participants with lower eGFR were older, tended to have higher SBP, TG, HbA1c, and Hcy levels, and more frequently to have hypertension, diabetes, and a history of antihypertensive and hypoglycemic medications.

Table 3-1 Baseline characteristics in participants by baseline eGFR

Characteristics	**eGFR ≥90 (n = 205)**	**eGFR 60 to 89 (n = 210)**	**eGFR <60 (n = 101)**	***P* value**
Age, years	64.33 ± 10.28	69.40 ± 10.72	74.73 ± 10.08	<0.001
Male, n (%)	102 (49.76)	119 (56.67)	48 (47.52)	0.118
Current smoking, n (%)	35 (17.07)	31 (14.76)	17 (16.83)	0.806
Current drinking, n (%)	12 (5.86)	13 (6.19)	9 (8.91)	0.574
SBP, mmHg	141.65 ± 21.87	143.20 ± 20.12	149.03 ± 20.14	0.012
DBP, mmHg	86.02 ± 12.64	85.10 ± 11.70	83.82±10.50	0.505
BMI, kg/m^2	23.73 ± 3.47	23.49 ± 3.06	23.70 ± 3.03	0.853
Blood test, mean (SD)				
TCH, mmol/L	4.25 ± 1.01	4.40 ± 1.06	4.21 ± 1.07	0.277
HDL-C, mmol/L	1.16 ± 0.30	1.12 ± 0.28	1.05 ± 0.28	0.009
LDL-C, mmol/L	2.61 ± 0.92	2.75 ± 0.91	2.57 ± 0.93	0.167
TG, mmol/L	1.27 ± 0.78	1.40 ± 0.77	1.38 ± 0.64	0.038
HbA1c, (%)	6.14 ± 1.38	6.10 ± 1.30	6.35 ± 1.25	0.005
Hcy, umol/L	10.49 ± 2.74	11.20 ± 3.41	14.10 ± 5.25	<0.001
eGFR, ml/min/1.73 m^2	106.65 ± 15.91	77.86 ± 7.67	46.92 ± 12.29	<0.001

Medical History, n (%)				
Hypertension, n (%)	113 (55.12)	125 (59.52)	84 (83.17)	<0.001
Diabetes, n (%)	41 (20)	39 (18.57)	43 (42.57)	<0.001
Ischemic stroke, n (%)	22 (10.73)	21 (10)	16 (15.84)	0.275
Any CAD, n (%)	14 (6.83)	30 (14.29)	24 (23.76)	<0.001
Medications, n (%)				
Antihypertensives, n (%)	96 (46.83)	101 (48.10)	75 (74.26)	<0.001
Antidiabetics, n (%)	36 (17.56)	33 (15.71)	37 (36.63)	<0.001
Antiplatelets, n (%)	17 (8.29)	21 (10)	17 (16.83)	0.069
Statins, n (%)	12 (5.85)	11 (5.24)	13 (12.87)	0.034

Categorical variables are shown as number (percentage); continuous variables as mean +/− standard deviation.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TCH, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated hemoglobin; Hcy, homocysteine; eGFR, estimated glomerular filtration rate; CAD, coronary artery disease.

Participant characteristics by IAC patterns are shown in **Table 3-2**. For the 440 participants with calcification, 189 (42.95%) showed predominantly intimal IAC patterns and 251 (57.05%) showed predominantly medial patterns of calcification. Compared to the intimal IAC group, patients with medial IAC were older, had higher SBP, HbA1c and Hcy levels, and lower level of eGFR.

Table 3-2 Baseline Characteristics in Participants by calcification patterns

Characteristics	**Medial**	**Intimal**	***P* value**

	(n = 251)	**(n = 189)**	
Age, years	73.36 ± 9.54	66.70 ± 9.87	<0.001
Male, n (%)	133 (53.2)	102 (53.97)	0.873
Current smoking, n (%)	34 (13.55)	36 (19.05	0.118
Current drinking, n (%)	13 (5.17)	15 (7.98)	0.235
SBP, mmHg	147.98 ± 21.85	141.19 ± 19.09	0.001
DBP, mmHg	85.36 ± 11.99	84.77 ± 11.35	0.705
BMI, kg/m^2	23.49 ± 3.25	23.76 ± 3.14	0.343
Blood test, mean (SD)			
TCH, mmol/L	4.24 ± 1.03	4.35 ± 1.06	0.402
HDL-C, mmol/L	1.10 ± 0.28	1.13 ± 0.27	0.282
LDL-C, mmol/L	2.62 ± 0.88	2.68 ± 0.96	0.504
TG, mmol/L	1.37 ± 0.71	1.38 ± 0.87	0.500
HbA1c, (%)	6.30 ± 1.42	6.13 ± 1.29	0.02
Hcy, umol/L	12.27 ± 4.44	10.89 ± 3.12	0.003
eGFR, ml/min/1.73 m^2	76.06 ± 25.66	88.62 ± 21.94	<0.001
Medical History, n (%)			
Hypertension, n (%)	186 (74.10)	108 (57.14)	<0.001
Diabetes, n (%)	72 (28.69)	42 (22.22)	0.126
Ischemic stroke, n (%)	32 (12.75)	19 (10.05)	0.374
Any CAD, n (%)	41 (16.33)	19 (10.05)	0.057
Medications %			

Antihypertensives, n (%)	156 (62.15)	93 (49.21)	0.007
Antidiabetics, n (%)	59 (23.51)	39 (20.63)	0.474
Antiplatelets, n (%)	31 (12.35)	15 (7.94)	0.134
Statins, n (%)	17 (6.77)	11 (5.82)	0.685

Categorical variables are shown as number (percentage); continuous variables as mean +/− standard deviation.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TCH, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated hemoglobin; Hcy, homocysteine; eGFR, estimated glomerular filtration rate; CAD, coronary artery disease.

3.1.3.2 Kidney function and IAC scores

Mean IAC scores were higher in the mildly reduced eGFR group and decreased eGFR group compared with the normal kidney function group (5.30 vs. 2.82; 3.88 vs. 2.82, p <0.001) (**Figure 3-3**). eGFR <60 ml/min/ 1.73 m^2 was associated with IAC scores in univariate logistic regression (OR 2.70, 95% CI 2.18–3.33; p <0.001). In multivariate analyses (**Table 3-3**), eGFR <60 ml/min/1.73 m2 remained independently associated with the IAC scores in all three models (OR, 2.43; 95% CI, 1.93–3.207; p < 0.001 in model 1; OR, 2.48; 95% CI, 1.92–3.19; p <0.001 in model 2; OR, 2.01; 95% CI, 1.50–2.71; p <0.001 in model 3).

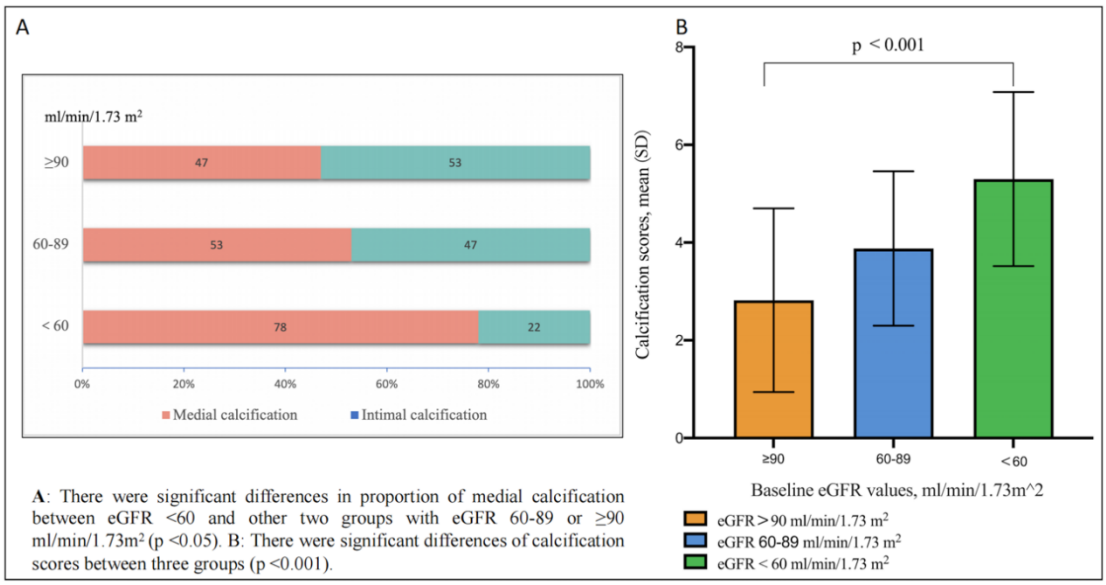

Figure 3-3 The degree and pattern of IAC in participants by baseline eGFR.

Table 3-3 Multiple analysis of eGFR and IAC scores

eGFR	Unadjusted	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
eGFR ≥90	Ref.	Ref.	Ref.	Ref.
eGFR 60 to 89	1.37 (1.22, 1.53) [a]	1.27 (1.07, 1.44)	1.32 (1.14, 1.52) [a]	1.34 (1.13, 1.59)
eGFR <60	2.70 (2.18, 3.30) [a]	2.43 (1.93, 3.07) [a]	2.48 (1.92, 3.19)[a]	2.01 (1.50, 2.71) [a]

[a] P <0.001

Model 1: adjusted for age and sex.

Model 2: further adjusted for body mass index (BMI), smoking status, alcohol use, SBP and DBP.

Model 3: further adjusted for CAD, history of stroke, history of hypertension, history of diabetes, TG, TC, LDL, HDL, HbA1c, Hcy, and the use of antihypertensives medication, hypertension medication, antiplatelets medication and statins medication

3.1.3.3. Kidney function and IAC patterns

As seen in Fig. 3, medial calcification was more prevalent in the lower eGFR group (eGFR <60 ml/min/1.73 m^2) than the other two groups with eGFR 60 to 89 and eGFR >90 ml/min/1.73 m^2 (78.72% vs. 53.65%, p <0.001; 78.72% vs. 47.78%, p < 0.001). No significant difference in the frequency of medial calcification was found between eGFR 60 to 89 and eGFR >90 ml/min/1.73 m^2 (p >0.05). Multivariate regression analyses (**Table 3-4**) demonstrated lower eGFR level was associated with higher prevalence of medial calcification in all three models (OR, 3.47; 95% CI, 1.92–6.28; p <0.001 in model 1; OR, 3.52; 95% CI, 1.85–6.71; p <0.001 in model 2; OR, 2.75; 95% CI, 1.36–5.53; p <0.001 in model 3).

Table 3-4. Multiple analysis of eGFR and medial calcification

eGFR	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
eGFR ≥90	Ref.	Ref.	Ref.	Ref.
eGFR 60 to 89	1.61 (1.09, 2.38) [a]	1.14 (0.75, 1.76)	1.27 (0.80, 2.02)	1.21 (0.74, 1.97)
eGFR <60	5.56 (3.24, 9.54) [a]	3.15 (1.76, 5.61) [a]	3.17 (1.69, 5.95) [a]	2.45 (1.23, 4.85) [a]

[a] P <0.05

Model 1: adjusted for age and sex.

Model 2: further adjusted for body mass index (BMI), smoking status, alcohol use, SBP and DBP.

Model 3: further adjusted for CAD, history of stroke, history of hypertension, history of diabetes, TG, TC, LDL, HDL, HbA1c, Hcy, and the use of antihypertensives medication, hypertension medication, antiplatelets medication and statins medication.

3.1.4 Discussion

In this study, we examined whether kidney function evaluated by eGFR was associated with the degrees and patterns of IAC. The results obtained showed that a lower eGFR was associated with higher degrees of calcification in the intracranial arteries after adjusting for demographic and cardiovascular risk factors. Compared to participants with normal kidney function and mildly reduced eGFR, patients with lower eGFR below 60 ml/min/1.73 m^2 had greater medial calcification in intracranial larger arteries.

Renal impairment has been reported to be related to arterial calcification in different populations. In a clinical study of 38 renal dialysis patients, the prevalence of vascular calcification increased from 39% at initiation to 92% during a mean follow-up of 16 years.[68] Similarly, Sedaghat et al.[12] found that a lower eGFR was associated with higher volumes of calcification in different vascular beds among community-dwelling individuals aged 45 or above. However, the effect estimates attenuated after adjusting for traditional cardiovascular risk factors. In the present study, based on hospitalized patients, lower eGFR was found to be prominently associated with the degree of IAC after adjusting for traditional risk factors, showing that arterial calcification does not only occur in specific types of blood vessels, but is a more systemic phenomenon.

Although the underlying mechanisms of decreased renal function on IAC are not fully understood, previous studies have suggested a specific contribution to the development of arterial calcification in patients with impaired renal function. Emerging evidence

shows that nontraditional risk factors, including uremic toxins, CKD-mineral and bone disease (CKD-MBD), oxidative stress, and inflammation, may contribute to the development of vascular calcification and cardiovascular disease in CKD patients.[69-72] Moreover, vascular calcification is not only considered a consequence of chronic kidney disease but may be a possible mechanism in the pathogenesis of chronic kidney disease. Several recent studies reported that renal function deteriorated more rapidly in subjects with higher degrees of vascular calcification, suggesting that arterial calcification might be associated with CKD progression, which may be explained indirectly by arterial stiffness exposing the glomerular capillaries to higher pulse pressure.[73,74]

IAC in large intracranial arteries is found not only in the tunica intima, but also in the tunica medial layers of arteries, which may differ with respect to clinical risk factors and outcomes. In this study, we found that IAC was dominated by medial calcification in 57% of patients. But interestingly, when we classified calcification types by renal function, medial calcification was predominantly detected (79.8%) in patients with eGFR <60 ml/min/1.73 m^2 compared with subjects with normal and mildly decreased kidney function, suggesting the deterioration of renal function may be one of the key factors causing the differentiation of calcification types. The relationship between decreased kidney function and calcification types remains controversial.[66,75-77] A study of patients with end-stage kidney disease found a high percentage of medial calcification in the inferior epigastric artery. Similarly, in studies of adolescents on

dialysis without traditional risk factors, medial calcification was found almost exclusively. However, several previous studies reported the coexistence of intimal and medial calcification, which may be attributed to the fact that many traditional atherosclerotic risk factors are more likely to be present in adult CKD patients.[78]

Decreased renal function is theoretically more likely to lead to medial calcification rather than intimal calcification, and the possible mechanisms are as follows: first, disturbances in calcium and phosphate metabolism due to decreased kidney function trigger abnormal mineral deposition on the medial layer of the arterial wall.[79,80] Second, previous studies showed medial calcification to be paralleled by significant higher in situ expression of proinflammatory markers, suggesting media calcification may be associated with local inflammation of the vascular wall.[81,82] Third, the kidney is one of the main sources of antioxidant enzymes, and increased oxidative stress in response to decreased renal function may further lead to medial vascular calcification.[83]

In the present study, we found a high prevalence of medial calcification among asymptomatic patients with eGFR <60 ml/min/1.73 m^2, suggesting medial calcification may begin at the early stage of kidney disease. Based on pathological studies,[55,63,84] intimal arterial calcification existed in advanced stage of atherosclerotic plaques and could be used as a marker of atherosclerosis. However, although medial calcification does not generally lead to atherosclerosis, given that cerebral artery stiffness caused by medial calcification was strongly associated with future cerebrovascular disease events

and collateral vessel formation, a comprehensive treatment strategy for patients with early renal impairment is needed to reduce the risk of future cerebrovascular events.

Compared to those with traditional cardiovascular risk factors such as hypertension or diabetes, the prevalence of IAC among patients with early renal impairment has been less studied. Understanding the connections between renal function and IAC patterns may arouse the clinicians' and researchers' interests and attention to the occurrence of cerebral artery calcification among patients with early renal impairment. Secondly, our previous studies, together with this study, demonstrated that intimal and medial calcification are two distinct entities in the risk factors and clinical correlations with arterial stiffness or atherosclerosis. Compared to intimal calcification as an indicator of atherosclerosis, medial calcification is more associated with calcium and phosphate homeostasis and loss of inhibition and matrix vesicles.[85,86] Therefore, considering the possible etiology of medial calcification and its close relationship with renal dysfunction, timely intervention of early renal dysfunction to adjust calcium and phosphate metabolism could be effective in preventing medial calcification. According to the updated literature, early physical exercise is helpful in moderating vessel flexibility. We plan to conduct a follow-up study to investigate the role of blood biomarkers in earlier detection and prediction of IAC in patients with early renal impairment. In the long run, we will investigate the effects of interventional rehabilitations in preventing arterial stiffness caused by medial calcification.

There are several limitations to our study. First, direct causal relationships cannot be

established, further longitudinal cohort studies could help identify the potential effect of impaired kidney function on the progression of IAC. Second, given the relatively preserved kidney function of the population included in this study, it is difficult to further classify those with eGFR <60 ml/min/1.73 m^2. Third, despite adjustment for some main potential confounders in analysis, serum phosphorus and calcium levels were not considered in this analysis. Serum phosphorus and serum calcium have been found to be associated with the risk of subclinical atherosclerosis in both the general population and CKD patients. Future studies are needed to investigate the role of calcium and phosphorus levels in the development of IAC. Lastly, a body of evidence has suggested that vitamin K status is associated with arterial calcifications, particularly in patients with end-stage renal disease (ESRD).[86-88] Considering relatively early and mild renal dysfunctions in this cohort of patients, the effects of vitamin K status were not investigated. Future studies are needed to clarify the relationship between vitamin K status and different patterns of IAC. Additionally, future research could explore the impact of interventions targeting renal function on calcification progression and refine current renal impairment categorization by subdividing patients into more specific eGFR ranges for a clearer understanding of the relationship between renal function and calcification development in the brain.

In conclusion, our findings demonstrated that impaired kidney function was independently associated with a higher degree of calcification in the intracranial arteries, especially medial calcification, which reflects that different underlying mechanisms

account for these two types of arterial calcification commonly identified in cerebral arteries.

Chapter 3-2:

Serum Phosphorus Concentration and Its Association with the Degree and Pattern of Intracranial Arterial Calcification

3.2.1 Introduction

Intracranial arterial calcification (IAC), a highly prevalent finding on head computed tomography (CT) scans, has been reported to be associated with ischemic stroke and cognitive impairment.[34,38,51] Previously considered as a proxy of atherosclerosis, vascular calcification shares some traditional cardiovascular risk factors with other atherosclerotic diseases, such as age, smoking, hypertension and diabetes.[89,90] In addition to these common risk factors, the occurrence of vascular calcification was also found to be related to calcium-phosphate imbalance mainly caused by impaired kidney function. As kidney function declines, the phosphate load increases and accumulates outside the bones, which inhibits the synthesis of 1,25-dihydroxy vitamin D and stimulates the secretion of parathyroid hormone (PTH). A lower concentration of 1,25-dihydroxy vitamin D and higher levels of PTH have been reported to accelerate artery calcification and increase cardiovascular disease (CVD) risk.[91-94] In coronary artery studies, a high level of serum phosphorus was shown to be linked with coronary artery calcification (CAC) in chronic kidney disease (CKD) patients as well as the general population.[79,85,95-97] However, to the best of our knowledge, little is known about the association between elevated serum phosphorus concentration (SPC) and IAC, especially among patients with preserved renal function.

Based on autopsy analysis, our histological study classified IAC as intimal or medial calcification according to its specific location in the vessel wall, which may differ with respect to risk factors and clinical outcomes.[55,66,75] Previous studies have shown that

phosphorus loading plays a key role in controlling arterial medial calcification in advanced CKD, however, until now, the effect of SPC on the patterns of IAC in patients with intact or mildly impaired renal function remains unclear.

There are insufficient data, however, to determine the association between high SPC and IAC, if so, earlier individual serum phosphorus management should be implemented to reduce the risk of IAC and further cerebrovascular disease. To fill this research gap, in the present study, we aimed to determine the effect of high SPC on IAC among patients with normal renal function or mild-moderate renal impairment.

3.2.2 Methods

This cross-sectional study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

3.2.2.1 Study Participants

We enrolled consecutive hospitalized patients who were admitted to the Department of Neurology and underwent brain CT scans between January 1, 2021, and March 1, 2022. Inclusion criteria were as follows: 1) age ≥18 years; 2) having performed brain CT within 1-mm slice thickness. The exclusion criteria were: 1) poor CT imaging quality; 2) insufficient clinical data for analysis; 3) history of chronic kidney disease; 4) receiving vitamin-K antagonists. Ethical approval for this study was obtained from the institutional review board of the Second Affiliated Hospital of Guangzhou Medical

University. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

3.2.2.2 Clinical and laboratory measurements

Demographic information (e.g., age and gender) and medical histories (coronary artery disease, stroke, hypertension, diabetes mellitus, hyperlipidemia, surgical history, medication history, smoking, and alcohol use status) were abstracted from the electronic medical records of the participants.

We measured the blood pressure, height and weight of study participants in the standard manner. Blood sampling was done in the morning after 8-12 hours of fasting duration. The serum phosphorous levels were measured by colorimetric analysis procedure, using the VITROS 5600 dry slide chemistry analyzer (Ortho Clinical Diagnostics, J&J) by the hospital laboratory. Total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglyceride (TG), hemoglobin A1c (HbA1c), homocysteine, eGFR, glycated hemoglobin, creatinine and homocysteine were also measured. We defined obesity as body mass index (BMI) ≥25 kg/m^2 according to the World Health Organization criteria. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic pressure ≥90 mmHg or a history of hypertension and/or use of antihypertensive medication. Diabetes mellitus (DM) was determined using a 75g oral glucose tolerance test, or HbA1c ≥6.0%, or a medical history of diabetes.

3.2.2.3 Image acquisition

The scan was performed by using a 64-row multidetector CT scanner without contrast administered. Axial images were acquired with the following parameters: 120 kVp, 170 mAs, 1-sec rotation time. Reconstructed CT images were independently evaluated by two readers with at least two years of experience in CT image interpretation, who were blinded to any clinical information of all the participants.

3.2.2.4 Assessment of IAC scoring

The visual grading method was used to assess the scores of IAC. Seven main intracranial arteries (bilateral internal carotid artery [ICA] C2-C7 segments, bilateral middle cerebral artery [MCA], bilateral vertebral artery [VA] V4 segments, and basilar artery [BA]) were assessed. As previously described, the severity of IAC was evaluated by the extent and thickness of calcification in individual cerebral arteries, a highest composite CT score of 0-2, 3-5 and 6-8 was classified as mild, moderate, and severe degree of IAC, respectively. The most calcified vessels were used for the final score.

3.2.2.5 Assessment of IAC patterns

The patterns of IAC were classified according to a previously established calcification scoring method. Points are scored as follows (**Figure 3-4**): Calcification circularity: absent (0 points); dots (1 point); <90 degrees (2 points); 90-270 degrees (3 points); 270-360 degrees (4 points). Calcification thickness: thick ≥1.5 mm (1 point); thin <1.5 mm (3 points). Calcification morphology: indistinguishable (0 points); irregular/patchy (1

point); continuous (4 points). The sum of the calcification scores, with a threshold of seven, was used to separate intimal (1 to 6 points) and medial calcifications (7 to 11 points).

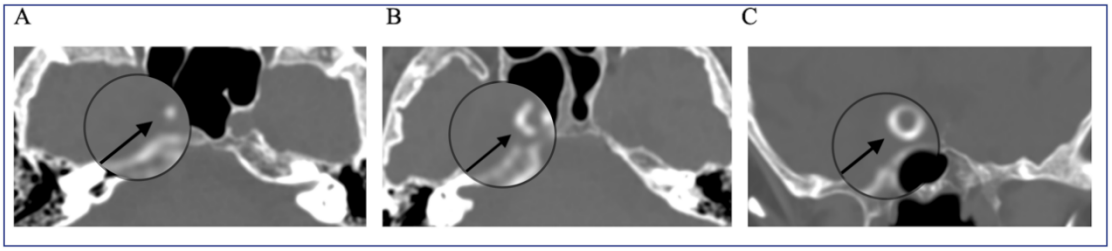

Figure 3-4 Examples of IAC scores and patterns on CT image. A: 1 point for extent and 1 point for thickness (mild); intimal calcification. B: 3 points for extent and 2 points for thickness (moderate); medial calcification. C: 4 points for extent and 2 points for thickness (severe); medial calcification.

3.2.2.6 Statistical Analysis

Kolmogorov-Smirnov formal test was used to assess the data distribution. Continuous variables with normal distribution were presented as mean and standard deviation (SD) or median and interquartile range (IQR) when non-normal distribution, categorical variables were presented as numbers and percentages. Our patients were divided according to the severity of calcification (mild, moderate, severe) and patterns of calcification (intimal and medial calcification). Characteristics of participants were compared using the chi-squared test for categorical data. Continuous variables were compared using the Kruskal-Wallis test. Logistic regression analysis was used to determine the association of SPC with degree and patterns of IAC, adjusting for age and sex (mode1), body mass index (BMI), smoking status, alcohol use, history of coronary artery disease, history of stroke, history of hypertension, history of diabetes

(model 2), and TG, TC, LDL, HDL, HbA1c, Hcy, and the use of antiplatelets medication and statins medication (model 3). A two-sided P value of less than 0.05 indicated statistical significance. Statistical analyses were performed using the SPSS software version 26 (IBM, Chicago, Illinois, USA).

3.2.3 Results

3.2.3.1 Participants

A total of 550 patients with thin-slice CT scans were initially included in the study. 20 patients were excluded due to the presence of artifacts on CT images, 13 patients were excluded due to insufficient clinical data for analysis and four on long-term vitamin-K antagonist therapy were excluded from the study, leaving 513 participants for analyses in the present study (**Figure 3-5**).

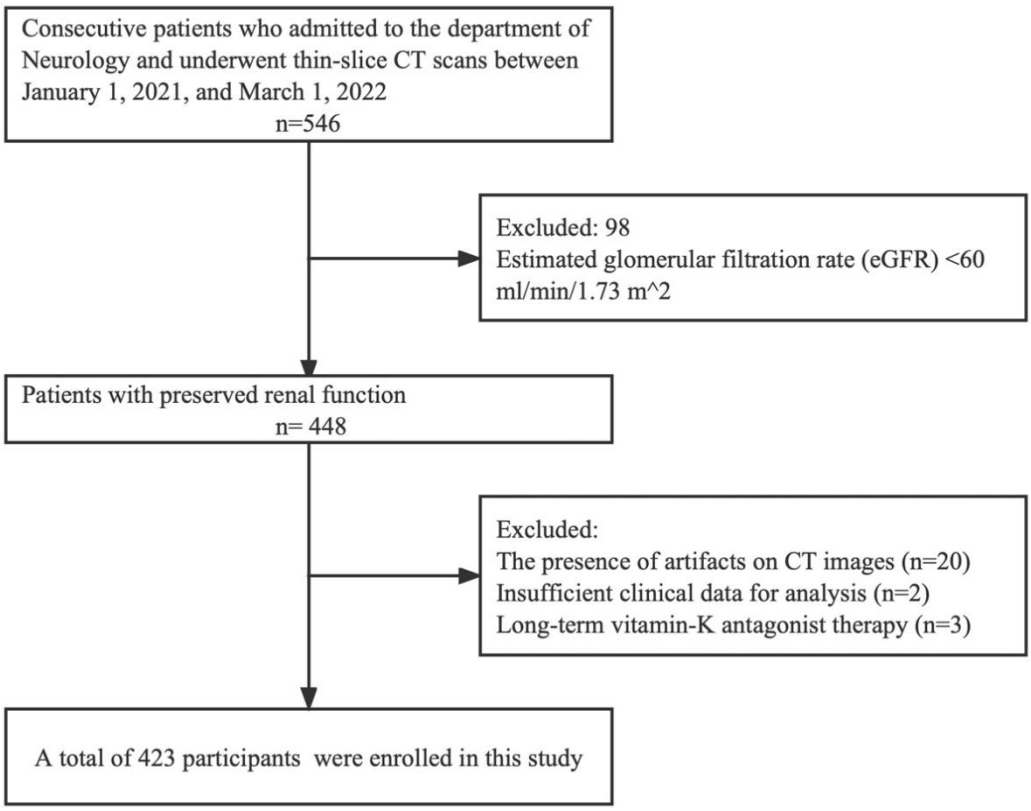

Figure 3-5 Flowchart of the study participants

The mean age of the population was 68.3 ± 10.3 years and 48% were female. Of these patients, 20% had a previous diagnosis of diabetes, 58% had hypertension, and 10% had a family history of coronary artery disease. The mean SPC was 1.07 ± 0.17 mmol/L and the IAC score was 4 (3-5).

3.2.3.2 Association between SPC and IAC scores

To investigate the association between baseline SPC and IAC scores, we divided subjects into 3 groups based on their IAC severity (mild, moderate, and severe). When compared to participants with mild IAC, those with moderate/severe IAC were considerably older, tended to have higher phosphorus and Hcy levels but lower eGFR values, and more frequently had hypertension and diabetes (P <0.001) (**Table 3-5**).

Multivariate regression analysis showed that higher SPC was associated with moderate/severe IAC in patients with eGFR ≥60 ml/min/1.73 m2 (OR, 1.27; 95% CI, 1.01–1.59; p <0.05 in model 3) and eGFR <60 ml/min/1.73 m2 (OR, 1.92; 95% CI, 1.04–3.57; p <0.05 in model 3) (**Table 3-7**).

Table 3-5. Characteristics of the patients evaluated, by IAC scores.

Characteristics	**Mild**	**Moderate**	**Severe**	***P* value**
	0-2	**3-5**	**6-8**	
	(n = 110)	**(n = 304)**	**(n = 99)**	
Age (years), mean ± SD	57.76 ± 8.36	69.64 ± 9.36	76.80 ± 9.49	<0.001
Male, n (%)	48 (43.63)	175 (57.57)	44 (44.44)	0.009
Current smoking, n (%)	21 (19.10)	51 (16.78)	9 (9.09)	0.103
Alcohol, n (%)	11 (10.00)	18 (5.92)	5 (5.05)	0.267
SBP (mmHg), mean ± SD	134.65 ± 18.70	145.31 ± 20.91	149.05 ± 20.98	<0.001
DBP (mmHg), mean ± SD	85.55 ± 12.43	85.46 ± 12.00	84.16 ± 10.87	0.629
BMI, kg/m^2, mean ± SD	23.69 ± 3.35	23.60 ± 3.02	23.63 ± 3.71	0.868
TCH (mmol/L), mean ± SD	4.35 ± 1.05	4.32 ± 1.03	4.18 ± 1.09	0.405
HDL-C (mmol/L), mean ± SD	1.15 ± 0.31	1.12 ± 0.29	1.09 ± 0.29	0.451
LDL-C (mmol/L), mean ± SD	2.72 ± 0.96	2.66 ± 0.91	2.59 ± 0.90	0.600
TG (mmol/L), mean ± SD	1.28 ± 0.63	1.38 ± 0.83	1.33 ± 0.60	0.794
HbA1c (%), mean ± SD	5.92 ± 1.24	6.18 ± 1.28	6.42 ± 1.51	<0.001
Hcy (umol/L), mean ± SD	10.23 ± 2.91	11.36 ± 3.43	13.47 ± 5.24	<0.001

eGFR (ml/min/1.73 m^2),	97.01	85.52	60.71	<0.001
median (IQR)	(85.25-107.25)	(74.45-97.90)	(48.15-74.30)	
Calcium, (mmol/L), mean ± SD	2.23 ± 0.10	2.23 ± 0.10	2.23 ± 0.14	0.987
Phosphorus (mmol/L), mean ± SD	1.07 ± 0.17	1.05 ± 0.17	1.15 ± 0.22	0.001
Hypertension, n (%)	41 (37.27)	203 (66.78)	76 (76.77)	<0.001
Diabetes, n (%)	16 (14.55)	71 (23.36)	36 (36.36)	0.001
Any CAD, n (%)	6 (5.45)	41 (13.49)	21 (21.21)	0.004
Antiplatelets use, n (%)	10 (9.09)	32 (10.53)	11 (11.11)	0.878
Statins use, n (%)	8 (7.27)	16 (5.26)	10 (10.10)	0.233

Categorical variables are shown as number (percentage); Normally distributed variables are expressed as mean ± SD; Non-normally distributed variables are expressed as median (IQR).

Table 3-7 Multiple analysis of SPC and IAC scores.

Characteristics	**eGFR (ml/min/1.73 m^2)**	**Unadjusted OR (95% CI)**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Moderate - severe IAC	**eGFR ≥ 60**	0.95 (0.83, 1.08)	1.23 (1.04, 1.45) [a]	1.29 (1.07, 1.55) [a]	1.27 (1.01, 1.59) [a]
	eGFR < 60	1.49 (1.08, 2.04) [a]	1.66 (1.14, 2.40) [a]	1.50 (1.01, 2.22) [a]	1.92 (1.04, 3.57) [a]

[a] $p<0.05$

Adjusting for age and sex (mode1), body mass index (BMI), smoking status, alcohol use, history of coronary artery disease, history of stroke, history of hypertension, history of diabetes (model 2), and TG,

TC, LDL, HDL, HbA1c, Hcy, and the use of antiplatelets medication and statins medication (model 3).

3.2.3.3 Association between SPC and IAC patterns

Participant characteristics by IAC patterns are shown in **Table 3-6**. Of the 437 participants with calcification, 188 (43.02%) showed predominantly intimal IAC patterns and 249 (56.72%) showed predominantly medial IAC. Compared to the patients in the intimal IAC group, patients with medial IAC were older (73.14 ± 9.56 vs. 66.65 ± 9.87, P <0.001), had higher SBP and Hcy values (p <0.05), and lower levels of eGFR (P <0.001), there was no statistically significant difference in SPC between the two groups (p=0.313). In the multivariate analysis (**Table 3-8**), no statistically significant association was found between SPC and medial calcification among patients with an eGFR ≥60 ml/min/1.73 m^2 (OR, 1.15; 95% CI, 0.98–1.34; p >0.05). Conversely, among patients with an eGFR <60 ml/min/1.73 m^2, a significant statistical correlation between the two variables was observed, even after adjusting for multiple confounding factors (OR 1.67, 95% CI 1.08-2.61; P <0.05).

Table 3-6. Baseline Characteristics in Participants by calcification patterns

Characteristics	**Medial**	**Intimal**	**P value**
	n = 249	**n = 188**	
Age (years), mean ± SD	73.14 ± 9.56	66.65 ± 9.87	<0.001
Male, n (%)	133 (53.41)	100 (53.19)	0.182
Current smoking, n (%)	34 (13.65)	36 (19.15)	0.559

Alcohol, n (%)	13 (5.22)	15 (7.98)	0.406
SBP (mmHg), mean ± SD	147.65 ± 22.07	141.27 ± 19.08	0.015
DBP (mmHg), mean ± SD	85.81 ± 12.21	84.68 ± 11.42	0.258
BMI, kg/m^2, mean ± SD	23.49 ± 3.25	23.73 ± 3.17	0.158
TCH (mmol/L), mean ± SD	4.25 ± 1.03	4.36 ± 1.06	0.250
HDL-C (mmol/L), mean ± SD	1.10 ± 0.29	1.14 ± 0.30	0.022
LDL-C (mmol/L), mean ± SD	2.63 ± 0.88	2.69 ± 0.95	0.352
TG (mmol/L), mean ± SD	1.38 ± 0.71	1.38 ± 0.87	0.250
HbA1c (%), mean ± SD	6.30 ± 1.42	6.12 ± 1.28	0.007
Hcy (umol/L), mean ± SD	12.29 ± 4.45	10.92 ± 3.12	<0.001
eGFR (ml/min/1.73 m^2), median (IQR)	76.11 (57.10-92.57)	87.99 (78.11-101.75)	<0.001
Calcium, (mmol/L), mean ± SD	2.22 ± 0.11	2.23 ± 0.11	0.580
Phosphorus (mmol/L), mean ± SD	1.09 ± 0.18	1.05 ± 0.17	0.319
Hypertension, n (%)	185 (74.30)	98 (56.00)	<0.001
Diabetes, n (%)	71 (28.51)	36 (20.57)	0.121
Any CAD, n (%)	41 (16.47)	15 (8.57)	0.083
Antiplatelets use, n (%)	31 (12.45)	15 (7.98)	0.132
Statins use, n (%)	17 (6.83)	11 (5.85)	0.457

Categorical variables are shown as number (percentage); Normally distributed variables are expressed as mean ± SD; Non-normally distributed variables are expressed as median (IQR).

Table 3-8. Multiple analysis of SPC and medial calcification.

Characteristics	eGFR (ml/min/1.73 m^2)	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Medial IAC	**eGFR ≥ 60**	1.00 (0.88, 1.13)	1.12 (0.98, 1.29)	1.14 (0.99, 1.32)	1.15 (0.98, 1.34)
	eGFR < 60	1.32 (0.98, 1.77)	1.45 (1.02, 2.04) *	1.36 (0.97, 1.91)	1.67 (1.08, 2.61) *

[a] $p<0.05$

Adjusting for age and sex (mode1), body mass index (BMI), smoking status, alcohol use, history of coronary artery disease, history of stroke, history of hypertension, history of diabetes (model 2), and TG, TC, LDL, HDL, HbA1c, Hcy, and the use of antiplatelets medication and statins medication (model 3).

3.2.4 Discussion

The main findings of the present study were summarized as follows: 1) Even in individuals with preserved renal function, a positive correlation was observed between elevated levels of serum phosphorus and the degree of IAC; 2) Higher SPC was independently associated with medial IAC only in patients with eGFR less than 60 ml/min/1.73 m^2.

Previous epidemiological data examining the association between serum phosphorus and the risk of artery calcification were mainly from the populations with advanced

CKD.[98-100] As impaired renal function progresses to advanced stages, with loss of nephrons and reduction in functional renal mass, the balance between dietary intake and excretion of phosphorus can be impaired, which may lead to a rise in SPC. In a clinical study of 205 patients with end-stage renal disease (ESRD) receiving dialysis, the prevalence of coronary artery calcification was found to be common, severe, and significantly related to ischemic CVD.[101] These findings have been extended to community subjects who did not have renal problems. In an earlier study, Park et al. reported a higher CAC score in those with SPC >3.9mg/dL compared to SPC <3.3mg/dL among healthy participants with eGFR >60 ml/min/1.73 m^2.[79,95]

In this study, higher SPC was associated with a risk of IAC in subjects with preserved renal function (eGFR >60 ml/min/1.73 m^2), suggesting that even relatively high SPC within the normal range may be a risk factor for cerebrovascular disease. However, to date, no studies have shown a benefit from active control of SPC. Furthermore, this is the first study showing an association between SPC with calcification in intracranial arteries, suggesting that the effects of serum phosphorus on arterial calcification occur as a systemic phenomenon in various blood vessels, rather than limited to specific types of blood vessels, such as previously reported coronary arteries.[79,95,102] Moreover, previously regarded as a proxy indicator of intracranial atherosclerosis, IAC has been reported to be a risk factor for ischemic stroke, white-matter disease or microbleeds, and cognitive impairment. Although the protective effect of lowering SPC in subjects with normal renal function is still unclear, the findings of this study suggest that it is

possible to reduce the occurrence of IAC by lowering the individual SPC and reducing the risk of future cerebrovascular disease.

IAC in large intracranial arteries was found not only in the tunica intima, but also in the tunica medial layers of arteries. In previous clinical studies, although the prevalence of medial calcification was found to be closely related to elevated calcium and phosphorus levels, most of the patients included in these studies were subjects with end-stage renal disease or receiving dialysis, and the extent to which serum phosphorus increases will lead to the differentiation of IAC into different patterns is still unclear. In this study, higher SPC was independently associated with medial IAC only in patients with eGFR less than 60 ml/min/1.73 m^2, which indicated that the imbalance of blood calcium-phosphorus levels driven by impaired renal function may be one of the main risk factors leading to the formation of medial calcification.

Several pathways and regulatory processes may play a critical role between phosphorus and IAC. Firstly, vascular smooth muscle cells (VSMCs) are key players in remodeling the extracellular matrix (ECM) of blood vessels.[6,103] Secondly, macrophages accelerate calcification by promoting the differentiation of vascular wall cells into osteoclast-like cells.[104,105] Thirdly, fibroblast growth factor 23 (FGF23), an endocrine hormone produced by osteocytes in bone, regulates serum phosphate level through its excretion and absorption.[106-108] Fourthly, reduction in Klotho expression caused by oxidative stress, may promote vascular calcification.[109] A growing body of research has shown

that serum phosphorus is closely related to vascular calcification in CKD and even the general population, thus, exploring the pathogenesis of high phosphate-induced cranial arterial calcification might help find earlier and more effective prevention and treatment of cerebrovascular disease.

The present study has several limitations. First, the cross-sectional design of the study cannot clarify a causal and temporal relationship between SPC and IAC, prospective cohort studies are required for this purpose. Second, one important potential confounder that we did not take into account is the level of FGF23, which are key regulatory hormone for serum phosphorus. Third, we could not include information about phosphorus intake because only a small percentage of participants provided information about supplement use.

In summary, our findings suggest that elevated serum phosphate levels were positively correlated with the degree of IAC, and this significant effect on medial IAC was only present in patients with impaired renal function (eGFR <60 ml/min/1.73 m^2).

Chapter 4:

The Hemodynamic Effects of Intracranial Artery Calcification

Chapter 4-1:

Brachial-ankle Pulse Wave Velocity is Associated with Intracranial Artery Calcification in Acute Stroke Patients

4.1.1 Introduction

Intracranial arterial calcification (IAC), an easily identified phenomenon on brain computed tomography (CT) imaging, have been established to be a risk factor of ischemic stroke or other brain diseases.[2,38] In our histological study based on 32 adult autopsy cases, depending on the location of the calcification within the vessel, IAC was categorized into two different forms: intimal and medial calcifications, intimal calcification is a marker of atherosclerosis and is exclusively associated with arterial luminal stenosis, while medial calcification seems to relate to increased arterial stiffness and reduced arterial compliance.[55,63]

Arterial stiffness is a complex process reflecting the adverse morphology and functional changes within the vascular wall. Arterial stiffness, assessed with the pulse wave velocity (PWV), has been suggested to be an independent predictor for both cardiovascular and fatal stroke events.[110-114] As a promising recommended measurement for

assessing arterial stiffness, brachial-ankle PWV (baPWV) is a developed noninvasive and nongraduating method for measuring arterial stiffness, especially in large clinical trials.[115,116] Several studies have validated the association between coronary artery calcification (CAC) and increased arterial stiffness measured by baPWV.[10,117,118] However, few studies have investigated the relationship between baPWV and IAC, and whether the degree of IAC could reflect the systemic arterial stiffness remains uncertain. To fill in this research gap, our study aimed to elucidate the association between IAC

and baPWV in patients with acute ischemic stroke (AIS).

4.1.2 Methods

4.1.2.1 Subjects

This study included consecutive patients between who were referred to our stroke center for ischemic stroke from January 2018 to April 2020. Patients who underwent both baPWV measurement and head CT scan within 7 days of the visit were enrolled in the study. Of these patients, we included patients who had AIS within 7 days of symptom onset; age of 18–80 years; who underwent head CT during the admission period and underwent magnetic resonance imaging (MRI) within 7 days of the admission; had complete clinical data or imaging information; no history of head injury or tumors. A total of 238 acute stroke patients with CT scans and baPWV measurement were initially included in this study. Patients with prior stroke, head injury or tumors, and renal disease or peripheral arterial disease were excluded from the study, after excluding 14 patients with image artifacts on CT and 8 patients with insufficient clinical data, 143 patients were enrolled in this study (**Figure 4-1**) All patients or immediate family members provided written informed consent. The study protocol has been approved by the Committee for Ethics of the Second Affiliated Hospital of Guangzhou Medical University.

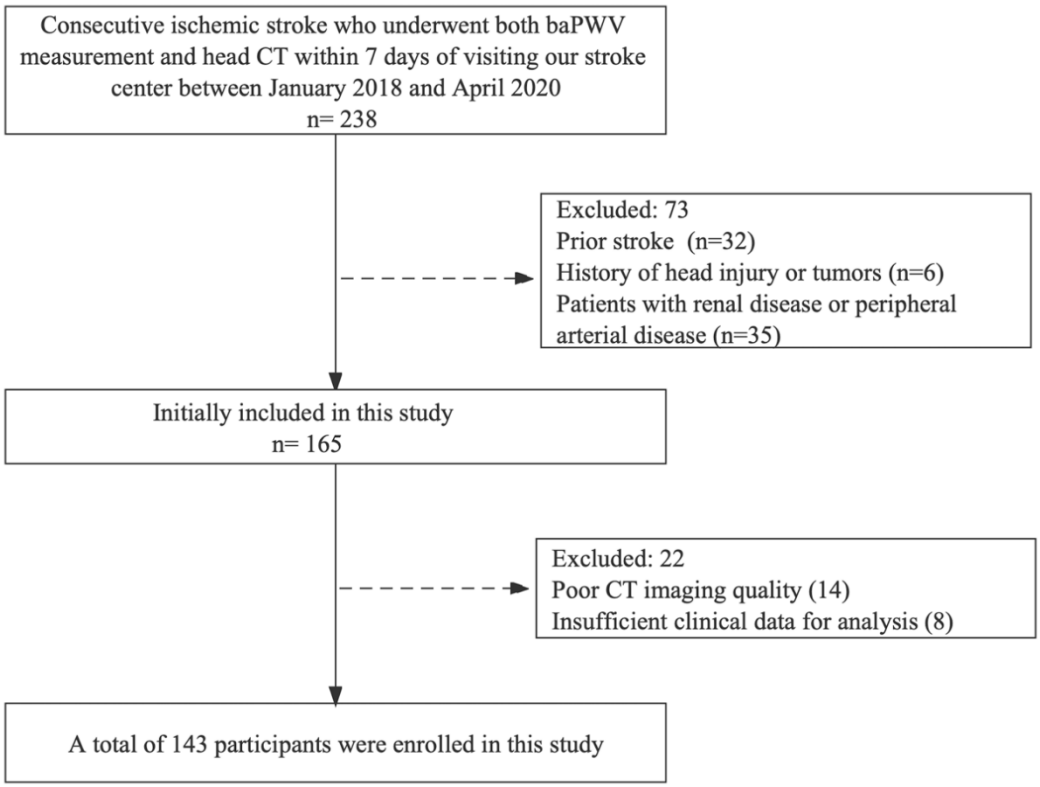

Figure 4-1 Flow chart of study participant exclusion criteria in this study

4.1.2.2 CT acquisition and processing

Imaging was performed by using a 64-row multidetector CT scanner without contrast administered. Patient's head to achieve a standard axial plane with tilting along the occipital-mental line, which cover every region from skull base to vertex. The presence of IAC, defined as hyperdense artery sign with a maximum density of more than 130 Hounsfield units. As previously established calcification scoring method described (**Figure 4-2**), the extents of calcifications were graded on a five-point scale as follows: absent (0 points), dots (1 point), <90◦ (2 points), 90–270◦ (3 points), and 270–360◦ (4 points). The calcification thickness was classified as follows: no calcification (0 points), 1 mm (1 point), 2 mm (2 points), 3 mm (3 points), and >3 mm (4 points). The overall

CT score of 0–2, 3–5, and 6–8 was considered as mild, moderate, and severe degree of IAC, respectively **(Table 4-1)**.[26,67]

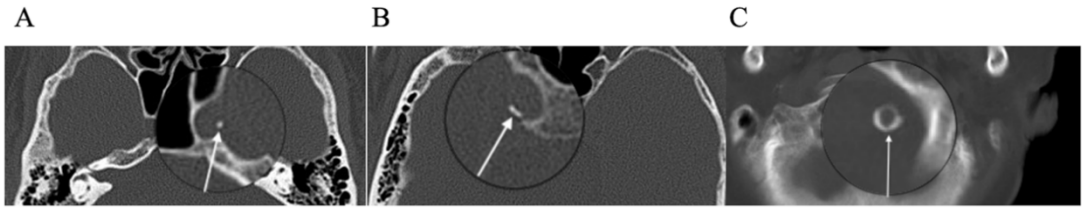

Figure 4-2 Examples of different degrees of intracranial artery calcification on a noncontrast CT image: A (mild degree of IAC): 1 point for extent and 1 point for thickness. B (moderate degree of IAC): LVA, 2 points for extent and 1 point for thickness. C (severe degree of IAC): 4 points for extent and 3 points for thickness.

Table 4-1 Grading scales for CT Scoring

Extent of calcification of intracranial artery		
Grade	0	No calcification
	1	Dot of calcification
	2	Crescentic area of calcification ＜90 of circumference
	3	Calcification 90-270 circumference
	4	Calcification 270-360 circumference
Thickness of calcification of intracranial artery		
Grade	0	No calcification
	1	Calcification 1 mm thick
	2	Calcification 2 mm thick
	3	Calcification 3 mm thick

	4	Calcification＞3 mm thick
Composite CT score of intracranial calcifications (sum of extent and thickness)		
Classification	Mild	0-2
	Moderate	3-5
	Severe	6-8

4.1.2.3 Pulse wave velocity measurement

For measuring baPWV, after a 10–15 min period of rest in supine position, bilateral baPWV was measured using an automated recorder (VP-1000; Colin Co. Ltd., Komaki, Japan). Oscillometric method was used to simultaneously measures the pulse waveform and arterial blood pressure of the bilateral brachial and posterior tibial arteries. The pulse wave transit time (Δmt) was calculated as the time spent for the waveform to travel from brachium to ankle. The transit distance (D, m) between the brachium and ankle was automatically calculated on the basis of body height. Thus, the baPWV on each side was calculated as baPWV = D/Δmt (cm/s). For analysis, the mean values of baPWV of both sides were used.

4.1.2.4 Statical analysis

baPWV was classified into quartiles. Within quartiles, data are presented as mean and standard deviation (SD) for continuous study characteristics, categorical variables and the presence of IAC are presented as percentages. Characteristics of participants were

compared according to the severity of arterial stiffness using the Kruskal-Wallis test for the continuous variables, and the chi-squared test for the categorical variables. Multiple logistic regression analysis was used to determine the independent correlation between baPWV and cerebral calcification. Adjustments were made to prevent the contribution of possible confounding biases. A p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using the SPSS software (version 26.0.0).

4.1.3 Results

143 subjects were included in this study. **Table 4-2** presents averages and percentages of the risk factors and clinical characteristics for the study population according to the quartiles of baPWV. Subjects with higher baPWV values tend to be older, diagnosed with hypertension, and tended to have higher cholesterol, blood levels of LDL-C, a higher percentage of subjects with IAC presence and IAC scores.

Table 4-2 Characteristics of study subjects

Characteristics	baPWV quartiles (cm/s)				p value
	<1491.5	1494.5-1723	1736.5-2120	≥ 2123	
	36	36	36	35	
Age, years	51.4±5.3	61.4± 5.4	63.8±5.8	70.9±6.4	<0.001
Male sex %	22 (61)	26 (72)	28 (78)	30(86)	0.012
Hypertension %	11 (30)	22 (61)	21 (57)	25 (69)	0.001

Diabetes %	5 (14)	8 (22)	7 (19)	10 (28)	0.358
CAD %	0	1 (3)	4 (11)	5 (14)	0.066
Current smoking %	13 (35)	12 (33)	9 (24)	9 (25)	0.313
Presence %	19 (53)	25 (69)	31 (86)	33 (94)	<0.001
Calcification scores	1.60±1.71	2.56±1.99	3.44±1.91	4.64±1.58	<0.001
BMI	24.0±2.5	23.4±2.7	23.3±2.7	22.6±3.2	0.262
SBP	143.7±23.4	144.6±19.7	149.5±24.0	142.8±18.5	0.814
Pulse pressure	53.7±14.8	56±13.3	61.1±18.9	61.8±14.4	0.069
TG	1.5±0.8	2.0±1.5	1.6±0.8	1.7±1.2	0.133
HbA1c	5.8±0.9	6.2±1.2	6.0±1.2	6.5±1.9	0.225
Cholesterol, mg/dl	4.6±1.4	4.6±0.98	4.3±1.2	3.9±1.1	0.004
LDL-C	2.9±0.8	2.9±0.9	3.0±0.99	2.4±0.9	0.002

Values are expressed as means ± standard deviations for continuous variables and as percentages for categorical variables.

Abbreviations: baPWV, brachial-ankle pulse wave velocity; CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; TG, Total Cholesterol; HbA1c, Hemoglobin A1c; LDL-C, Low-density lipoprotein cholesterol.

Table 4-3 presents the results of logistic regression analysis of baPWV and IAC presence. Quintiles 3–4 of baPWV was associated with IAC in univariate logistic regression ($p < 0.01$). The IAC score ratios (95% confidence intervals) were 8.21 (2.39, 28.20) and 14.3 (2.97, 69.05). After an additional adjustment for age and hypertension,

the correlation between baPWV and IAC presence became insignificant (P >0.05).

Table 4-3 Multiple analysis of baPWV and IAC presence.

baPWV, cm/s	N	Presence n (%)	No adjusted OR (95% CI)	P value	adjusted OR (95% CI)	P value
Quartile						
Q1 (<1491.5)	36	19 (53)	Ref.		Ref.	
Q2 (1494.5-1723)	36	25 (69)	2.75 (1.03, 7.38)	0.191	0.53 (0.14, 1.96)	0.84
Q3 (1736.5-2120)	36	31 (86)	8.21 (2.39, 28.20)	0.005	1.18(0.27, 5.22)	0.82
Q4 (≥ 2123)	35	33 (94)	14.3 (2.97, 69.05)	0.001	1.23 (0.15, 9.82)	0.34

Adjusted for age and hypertension.

Abbreviations: baPWV, brachial-ankle pulse wave velocity; CI, confidence interval; OR, odds ratio.

The logistic regression analysis results are presented in **Table 4-4**. Compared to the bottom quartile, odds ratios (95% confidence interval) of IAC score for the 2–4 quartiles were 1.35 (1.04–1.74), 1.72 (1.31–2.26), and 2.54 (1.82–3.54), respectively. After an additional adjustment for age and hypertension, the adjusted odds ratio for the IAC scores was 1.61 (1.06–2.45; P = 0.025) in the top quartile of baPWV compared with those in the bottom quartile.

Table 4-4 Multiple analysis of baPWV and IAC scores.

baPWV, cm/s	N	scores	Nonadjusted OR (95% CI)	P value	adjusted OR (95% CI)	P value

Quartile						
Q1 (<1491.5)	36	1.40±1.61	Ref.		Ref.	
Q2 (1494.5-1723)	36	2.97±1.64	1.35 (1.04, 1.74)	0.02	1.03 (0.76, 1.39)	0.875
Q3 (1736.5-2120)	36	3.53±2.01	1.72 (1.31, 2.26)	<.001	1.35(1.18, 1.54)	0.152
Q4 (≥ 2123)	35	4.33±2.04	2.54 (1.82, 3.54)	<.001	1.61 (1.06, 2.45)	0.025

Adjusted for age and hypertension.

Abbreviations: baPWV, brachial-ankle pulse wave velocity; CI, confidence interval; OR, odds ratio.

4.1.4 Discussion

In this study, not only was IAC more likely to be present in patients with higher baPWV, but the degree of IAC increased with increasing severity of artery stiffness. After adjustment for age and hypertension, only severe artery stiffness was correlated with a greater extent of IAC compared to none and mild artery stiffness, suggesting that age-based risk factors may mediate the association between IAC and baPWV.

IAC, previously regarded as a proxy for intracranial atherosclerosis (ICAS), is an easily identifiable entity on plain head computed tomography scans. The highest prevalence of IAC is found in intracranial internal carotid artery (60–80%), followed by vertebral artery (17–35%), compared to basilar artery (2.5–7%) and middle cerebral artery (5%).[34] In the past two decades, using advanced imaging technologies, the characteristics and clinical impacts of IAC were explored. In terms of the clinical significance of IAC, several recent studies have demonstrated the value of IAC as a

predictor of first-ever and recurrent ischemic stroke risk, and the association between IAC and cognitive disorder has also been studied in recent years.[3,119] In addition, based on autopsy analysis and high-resolution magnetic resonance imaging (HR-MRI), we classified IAC as intimal or medial calcification determined by the locations of calcification within the artery layers, and demonstrated that the two distinct morphological patterns of IAC may represent different pathological processes that have distinct clinical outcomes.[55,63]

Although there have been multiple studies on CAC, relatively little evidence has verified the correlation between IAC and PWV. PWV reflects functional changes in vascular compliance, while vascular calcification may be related to morphological changes in arterial stiffness. Our results showed that there is a positive relationship between these two parameters of arterial stiffness, which is consistent with previous findings.[9,117,120] In a Chinese study, Zhang et al. reported that elevated arterial stiffness was independently associated with intracranial large artery disease, presented as intracranial stenosis or calcification.[8] Park et al.[121] firstly reported a positive association of elevated baPWV with the degree of CAC among ischemic stroke patients, however, only 67 patients were included in the study.

In this study, after an additional adjustment for age, the association between baPWV and presence of IAC became insignificant. Following aging, multiple comorbid risk factors, which are invariably high prevalence among the elderly, accelerate the

atherosclerotic process. Age related changes in the arterial structure and large artery remodeling may reduce wall stress and further leading to arterial stiffness. Vascular calcification is also strongly related to aging, several previous studies that have used several methods to measure the severity of calcification have demonstrated a trend for IAC to increase with age.[122] Coronary calcification has been found in up to 89% of the general older population. Furthermore, prevalence of up to 83% for the extracranial carotid arteries in the general older population. Consistent with our autopsy-based histopathological study, the present study found a prevalence of 76% for IAC among patients with ischemic stroke.[84]

IAC is found not only in the tunica intima, but also in the tunica medial layers of arteries.[13,66] Unlike intimal calcification, medial calcification is rarely related with progressive atherosclerotic lesions, instead is relevant to the decreased vascular compliance. IAC calcifications are predominantly medial, our recent study examining intracranial arteries shown that medial arterial calcification accounted for up to 60% of all IAC tyees and 71% of intracranial internal carotid artery calcification. In a cohort study of 1132 subjects, predominant intimal pattern was found in 30.9% and medial calcification in 46.9%.[90] In this study, we could not differentiate whether the calcium is in the intima or the media due to the CT parameters and sample size. However, this study found that IAC is significantly related to arterial stiffness, which may be explained by IAC calcifications are mainly medial.

In this study, we used the baPWV as a means of measuring artery stiffness. Although cfPWV is considered the gold standard of measuring the stiffness, baPWV is also widely used in clinical practice as a measurement of both the central and peripheral arterial stiffness.[123-125] However, due to the lack of methods to directly measure the cerebral arterial stiffness, only a small number of studies have discussed the relationship between IAC and cerebral arterial stiffness. Based on our previous method using a noninvasive technical to evaluate carotid–cerebral PWV (ccPWV),[126,127] further studies are needed to investigate the effect of IAC on cerebral artery stiffness among stroke patients.

There are also some limitations to consider with these findings. Firstly, as a cross-sectional study, causal correlation between baPWV and IAC cannot be confirmed. Secondly, in this study, we only used baPWV to measure arterial stiffness, and further work is needed to explore the association between cfPWV or ccPWV and IAC. Thirdly, the IAC scores was determined by visual system rather than by an automated measurement. Lastly, in this study, due to the small sample size, we only adjusted for age and hypertension to assess independent association between baPWV and IAC, there may also have residual or unmeasured confounders that may affect the results of the study. Future cohort studies with larger sample sizes are needed to explore the impacts of other potential factors on the relationship between baPWV and IAC.

4.1.5 Conclusion

In conclusion, arterial stiffness as defined by an increased baPWV was positively associated with the degree of IAC in patients with acute ischemic stroke, suggesting the severity of IAC may also be a marker of peripheral or systemic arterial stiffness.

Chapter 4-2:

Cerebral Arterial Stiffness as Measured Based on the Pulse Wave Velocity is Associated with Intracranial Artery Calcification in Patients with Acute Stroke

4.2.1 Introduction

Intracranial artery calcification (IAC) is a relatively frequent finding on brain computed tomography (CT) in both the general population and patients with ischemic stroke. Our previous clinical studies found a high prevalence of IAC among the general population and patients with stroke or transient ischemic attack.[2,63] Consistent with its high prevalence, several recent population-based studies found increases in the risks of stroke and a poor outcome after stroke.[32,33,51,128,129] IAC can present in either the intimal or medial layers of the arteries; our previous pathological study also found that intimal calcification was often close to the internal elastic lamina and nearly always was within atheromatous plaques, while medial artery calcification might be linked to the increased stiffness of the vessel.[55,63]

The arterial stiffness is influenced by functional and structural changes in the vascular wall, which can mostly be assessed using pulse wave velocity (PWV). Among PWV measurements, although carotid–femoral PWV (cfPWV) is currently considered the gold standard for noninvasive measurements of arterial stiffness, brachial–ankle PWV (baPWV) has become the most widely used parameter of large-artery compliance in previous clinical studies and clinical practice.[130-132] However, baPWV and cfPWV measurements cannot be used to directly assess cerebral arterial stiffness. The measurement of carotid–cerebral PWV (ccPWV), which is simple and noninvasive, has recently become generally available as a measure of cerebral artery stiffness. Moreover,

our previous research has also found a strong correlation between ccPWV and baPWV.[127]

Despite numerous recent studies verifying the significant correlation between IAC and arterial stiffness by determining baPWV, few have addressed the association between IAC and cerebral arterial stiffness. In the present study, based on our previous method that used a novel original time and distance assessment technique to directly evaluate the human ccPWV, we aimed to determine the effect of IAC on cerebral artery stiffness among patients with stroke.

4.2.2 Methods

4.2.2.1 Subjects

Between September 2018 and June 2020, 146 patients with ischemic stroke who underwent both ccPWV measurement and head CT within 7 days of visiting our stroke center were enrolled in the study.

We included patients who were admitted within 7 days of acute ischemic stroke symptom onset, were 18–80 years old, underwent CT and magnetic resonance imaging (MRI) within 7 days of admission, had complete clinical data or imaging information, and had no history of head injury or tumors. Among the 206 consecutive patients initially examined or considered for inclusion in this study, 32 were excluded because of incomplete or no data on temporal windows for ccPWV measurements, and 28 for

not having undergone noncontrast head CT. All patients or their immediate family members provided written informed consent. The study protocol was approved by the clinical ethics committees of the participating hospitals (IRB No. 2021-hs-23).

4.2.2.2 CT data acquisition and processing

Imaging was performed using a 64-row multidetector CT scanner without contrast agent. All unenhanced head-tilted brain scans were performed in the axial mode with tilting along the occipitomeatal line, which covered the region from the skull base to the vertex. Axial images were acquired with the following parameters: 5-mm slice thickness, 120 kVp, 170 mAs, and 1-sec rotation time.

A visual grading method was used to assess IAC scores.[67] The presence of IAC was defined as a hyperdense artery sign with a peak density exceeding 130 Hounsfield units. Previously established calcification scoring methods that described the severity of IAC were evaluated by grading the values (extent and thickness) of individual cerebral arteries. The extents of calcifications were graded on a five-point scale as follows: absent (0 points), dots (1 point), <90° (2 points), 90°–270° (3 points), and 270°–360° (4 points).

The calcification thickness was classified as follows: no calcification (0 points), 1 mm (1 point), 2 mm (2 points), 3 mm (3 points), and >3 mm (4 points). Peak composite CT scores (sum of the extent and thickness) of 0–2, 3–5, and 6–8 were classified as mild, moderate, and severe degrees of IAC, respectively (**Figure 4-3**).

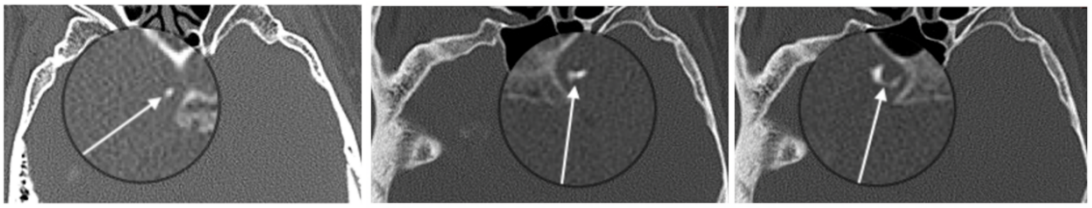

Figure 4-3 Examples of IAC scores in computed tomography. According to Babiarz's visual grading scales, IACs were graded as follows: (A) 1 for extent and 1 for thickness (arrow), (B) 2 for extent and 2 for thickness (arrow), and (C) 3 for extent and 3 for thickness (arrow). IAC, intracranial artery calcification.

4.2.2.3 Measurement of cerebral arterial stiffness

As previously described, bilateral ccPWV measurements were performed by two experienced operators using two-channel (2 and 4MHz) transcranial Doppler sonography (TCD-2000M, Beijing Chioy Medical Technology, Beijing, China) after a 10–15 min period of rest in the supine position. The 2-MHz ultrasound probe was placed on the temporal window to measure the cerebral blood flow velocity (CBFV) of the middle cerebral artery; the 4-MHz probe, with the angle fixed at 30°, was placed beside the thyroid notch in the neck of the patient to measure the CBFV of the common carotid artery. The mean pulse wave transmission time (Δmt) for ten consecutive cardiac cycles was automatically measured by the arterial pulse wave analysis system. The transit distance (D, in meters) traveled by the pulse wave was calculated by measuring the body surface distance between thetwo recording sites (D1, in meters) plus cosine (30°) of the detecting depth for the common carotid artery (D2, in meters); namely, D=D1+D2×cosine (30°). ccPWV on each side was therefore calculated as

ccPWV=D/Δmt (in centimeters/second). The reproducibility, reliability, and validity of ccPWV measurements were determined in our previous studies.[126,133,134]

4.2.2.4 Statistical analysis

Data are presented according to ccPWV quartiles. Within the quartiles, continuous clinical characteristics are presented as mean and standard-deviation values, categorical variables are presented as counts and percentages, and IAC scores are expressed as median (interquartile range) values. The characteristics of the participants were compared according to arterial stiffness severity using the chi-square test for the categorical variables. Continuous variables were compared across quartiles using the Kruskal-Wallis test. Multiple logistic regression analysis was used to examine the independent relationship between ccPWV and the degree of IAC. All reported p-values are based on a two-sided level of significance of less than 0.05. Statistical analyses were performed using SPSS software (version 26.0.0; IBM Corp., Armonk, NY, USA).

4.2.3 Results

After applying the inclusion and exclusion criteria, 146 patients were included. The baseline demographics and clinical characteristics according to ccPWV quartile are listed in **Table 4-5**.

Table 4-5 Characteristics of study subjects

	ccPWV quartiles

Characteristics	1st (n = 37)	2nd (n = 37)	3rd (n = 36)	4th (n = 36)	p value
	<716	718.05-847.15	847.8-956.6	≥ 965.85	
Age, years	54.4±8.8	62.5±6.2	65.1±11.1	68.0±9.9	＜0.001
Male sex	21 (58)	27 (75)	27 (75)	31 (88)	0.325
Hypertension	11 (30)	19 (53)	20 (54)	29 (81)	0.007
Diabetes	8 (22)	4 (11)	8 (22)	10 (28)	0.565
CAD	0	3 (8)	3 (8)	4 (11)	0.287
Current smoking	12 (32)	11 (31)	12 (32)	10 (28)	0.934
Presence %	20 (54)	28 (76)	30 (83)	32 (89)	＜0.001
calcification scores	1.40±1.61	2.97±1.64	3.53±2.01	4.33±2.04	＜0.001
BMI	22.5±2.4	23.97±2.6	24.3±3.4	22.6±2.99	0.076
Systolic BP, mm Hg	143.9±21.7	146.4±19.4	143.9±19.2	146.2±25.3	0.900
Pulse pressure	53.6±15.9	58.4±15.9	58.0±15.8	62.4±14.9	0.087
TG	1.5±0.8	1.9±1.2	1.9±0.9	1.6±1.5	0.763
HbA1c, %	5.8±0.9	6.3±1.5	6.1±1.2	6.2±1.5	0.266
Cholesterol, mg/dl	4.3±1.0	4.7±0.9	4.2±0.8	3.8±1.1	0.075
LDL-C	2.9±0.8	3.1±0.8	2.7±0.8	2.4±0.8	0.188

Values are expressed as means±standard deviations for continuous variables and as percentages for categorical variables.

Abbreviations: baPWV, brachial-ankle pulse wave velocity; CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; TG, Total Cholesterol; HbA1c, Hemoglobin A1c; LDL-C, Low-density lipoprotein cholesterol.

When compared with participants with the lowest ccPWV values, those with higher ccPWV values were more likely to be older, be diagnosed with hypertension, have higher blood cholesterol levels, have IAC, and have higher IAC scores ($p<0.01$). IAC was present in 74.6% of the population (in 51.3%, 75.7%, 83.3%, and 88.9% of those in quartiles 1–4, respectively; $p<0.01$).

The logistic regression analysis results are listed in **Table 4-6**. There was a significant positive correlation between ccPWV and IAC in the logistic regression analysis ($p<0.05$). After additionally adjusting for age and hypertension, the correlation between the quartiles 2–4 of ccPWV and the presence of IAC became insignificant, as well as in the analysis that included ccPWV as a continuous variable ($p>0.05$). **Table 4-7** lists the results of the logistic regression analysis between ccPWV and calcification scores. The odds ratio (95% confidence interval) values for the IAC scores when comparing quartiles 2–4 of ccPWV with quartile 1 were 1.42 (1.11–1.83), 1.71 (1.30–2.25), and 2.24 (1.65–3.02), respectively ($p<0.05$). After an additional adjustment for age and hypertension, there was also a significant correlation between quartiles 3 and 4 of ccPWV and IAC scores. The odds ratio (95% confidence interval) values for the IAC scores were 1.78 (1.28–2.50) ($p=0.001$) in quartile 4 of ccPWV and 1.45 (1.07–1.95) ($p<0.05$) in quartile 2 compared with quartile 1. ccPWV was also independently associated with IAC when it was included in the model as a continuous variable ($p=0.001$).

Table 4-6 Multiple analysis of ccPWV and calcification presence.

ccPWV, m/s	N	Presence, N (%)	Non-adjusted OR (95% CI)	P value	adjusted OR (95% CI)	P value
Quartile						
Q1 (＜716)	37	19 (54)	Ref.		Ref.	
Q2 (718.05-847.15)	37	28 (78)	2.947 (1.05, 8.25)	0.040	1.49 (0.46, 4.84)	0.50
Q3 (847.8-956.6)	36	30 (83)	4.21 (1.40, 12.65)	0.010	1.70 (0.50, 5.89)	0.40
Q4 (≥ 965.85)	36	32 (89)	6.74 (1.96, 23.14)	0.002	2.02 (0.50, 8.28)	0.33

Adjusted for age and hypertension.

Abbreviations: ccPWV, carotid-cerebral pulse wave velocity; CI, confidence interval; OR, odds ratio.

Table 4-7 Multiple analysis of ccPWV and calcification scores.

ccPWV, m/s	N	scores	Non-adjusted OR (95% CI)	P value	adjusted OR (95% CI)	P value
Quartile						
Q1 (＜716)	37	1.40±1.61	Ref.		Ref.	
Q2 (718.05-847.15)	37	2.97±1.64	1.42 (1.11, 1.83)	0.006	1.22 (0.91, 1.63)	0.176
Q3 (847.8-956.6)	36	3.53±2.01	1.71 (1.30, 2.25)	＜.001	1.45 (1.07, 1.95)	0.015
Q4 (≥ 965.85)	36	4.33±2.04	2.24 (1.65, 3.02)	＜.001	1.78 (1.28, 2.50)	0.001

Adjusted for age and hypertension.

Abbreviations: ccPWV, carotid-cerebral pulse wave velocity; CI, confidence interval; OR, odds ratio.

4.2.4 Discussion

This study was the first to find that the degree of cerebral arterial calcification was correlated with cerebral arterial stiffness as measured by ccPWV independently from other conventional risk factors in patients with acute ischemic stroke.

PWV, which is virtually synonymous with arterial stiffness for many biomedical professionals, reflects functional and structural changes in the vascular wall compliance. Although cfPWV is considered the gold standard of stiffness measurements, baPWV is widely used in clinical practice due to its simplicity. However, few studies have specifically examined the association between IAC and cerebral arterial stiffness due to limitations when measuring the PWV of cerebral arteries. In the present study, we used a novel time and distance assessment technique based on our previous method to directly measure ccPWV in humans. Compared with baPWV and other PWV measurements, ccPWV could be used to directly assess cerebral arterial stiffness, which may be useful for future studies of intracranial atherosclerotic disease.

In this study, after adjusting for age and hypertension, arterial stiffness as measured using ccPWV was not associated with the presence of IAC, but with the IAC score. This inconsistency can be explained by the high prevalence of IAC in the elderly population; furthermore, the presence of calcification does not necessarily reflect IAC severity. Several previous studies found that vascular calcification was strongly related to aging.[135,136] In coronary artery disease, coronary artery atherosclerosis and

calcification have been observed in up to 89% in the elderly population. Furthermore, a high prevalence of calcification has also been found in the aortic arch and extracranial carotid arteries of the elderly.[137-139] Consistent with these studies, our previous studies found that the IAC prevalence exceeded 70% among the general population and patients with stroke or transient ischemic attack.

The arterial stiffness and vascular calcification are independent predictors of cardiovascular morbidity and mortality, and several previous studies have found a significant correlation between them.[10,118,120,140] Park et al.[121] found that increased baPWV was closely associated with the degree of cerebral arterial calcification in patients with acute ischemic stroke; however, only 67 patients were included in that study. In a study in China, Zhang et al.[8] also found that increased arterial stiffness was independently associated with intracranial large-artery disease, which presented as intracranial stenosis or calcification. The findings of this research were consistent with those of previous studies on the association between measures of arterial stiffness and vascular calcification.

Several possible mechanisms may be responsible for the relationship between ccPWV and IAC. First, unlike intimal calcification, medial calcification is rarely related to progressive atherosclerotic lesions, but instead to decreased vascular compliance.[13,90,141] Moreover, IACs are predominantly located medially, and we have previously examined intracranial arteries and found that medial arterial calcification

accounted for about 60% of all calcifications and 71% of intracranial internal carotid artery calcifications. Second, IAC and arterial stiffness are known to be independent risk factors for cardiovascular disease. Moreover, these two pathological processes reinforce each other, generating a vicious circle in which endothelial and smooth-muscle cells play key roles.[7] Third, cerebral artery stiffness and IAC share several risk factors including age, sex, hypertension, BMI, and hypercholesterolemia, which are the foundations for linking IAC to cerebral arterial stiffness. This was the first study to use ccPWV to measure cerebral arterial stiffness, and we systematically addressed the correlation between IAC and cerebral arterial stiffness.[31,40]

PWV is a measure of arterial stiffness, which is an independent predictor of cardiovascular events and mortality. Increased PWV has been associated with various cerebrovascular diseases, including cerebral small vessel disease, white matter hyperintensities, and cognitive decline. IAC have been found to be associated with increased PWV, which may contribute to the development and progression of cerebrovascular diseases by increasing arterial stiffness. In addition, IAC have also been associated with brain changes and diseases, such as stroke, cognitive decline, cerebral small vessel disease. Although the exact mechanisms underlying the relationships between IAC, hemodynamic measures, and brain changes or diseases are not fully understood, based on the above information, we speculate that IAC may contribute to the development and progression of cerebrovascular diseases by causing vascular narrowing, reducing cerebral blood flow, and increasing arterial stiffness.

Further prospective studies with larger sample sizes are needed to clarify the relationship between IAC, hemodynamic measurements, and brain changes or diseases.

There were also some limitations to these findings that must be considered. First, as a cross-sectional study, this study could not clarify a temporal relationship between IAC and cerebral arterial stiffness measured using ccPWV. Second, the IAC scores were determined using a visual system rather than an automated measurement process. Third, given the relatively small sample, it was not possible to conduct a subgroup analysis to assess the associations among middle-aged and elderly patients with stroke. Fourth, the body surface distance does not accurately represent the distance of the corresponding artery because the terminal internal carotid artery is circuitous. Fifth, due to the sample-size limitations in this study, we only adjusted for age and hypertension to assess the independent association between ccPWV and IAC, and so there may have also been residual or unmeasured confounders that affected the study results.

In conclusion, we found that ccPWV was positively related with the degree of IAC in patients with acute ischemic stroke. Further longitudinal cohort studies may help to identify the potential role of IAC in the progression of cerebral arterial stiffness.

Chapter 5. Conclusions and Future Directions

5.1 Conclusions

Stroke is the second leading cause of death and the third leading cause of disability worldwide, following heart disease. Strokes can result from various mechanisms that include intracranial atherosclerosis, one of the most common causes of ischemic stroke worldwide. Intracranial arterial calcification (IAC), although not all atherosclerotic, is often considered diagnostic of atherosclerosis. In the past few years, several studies have shown a significant association between IAC and first-ever ischemic stroke risk. Moreover, the value of IAC as a predictor of recurrent stroke risk was also demonstrated in these studies. However, it is important to note that the association between calcification and stroke has not gone undisputed. In chapter 2, we conducted a meta-analysis of existing studies of IAC and ischemic stroke, demonstrated that the presence of IAC was identified as an independent risk factor for ischemic stroke occurrence and recurrence.

In the serial hospital-based studies, we investigated the hemodynamic effects and underlying metabolic mechanisms of intracranial arterial calcification as an imaging biomarker of stroke based on two hospital databases. In chapter 3-1, we demonstrated that impaired kidney function is independently associated with higher degrees of calcification in the intracranial arteries, especially medial calcification. This is significant as it reflects a specific contribution of kidney impairment to the development and differentiation of IAC. In chapter 3-2, we demonstrated that a higher-than-normal level of serum phosphorus was positively correlated with the degrees of IAC in patients

with preserved renal function or mild-moderate renal impairment, which highlights the importance of early management of serum phosphorus concentrations (SPC) for the prevention of IAC and further cerebrovascular disease, and the significant effect of SPC on the medial IAC was only present in patients with impaired renal function (eGFR <60 ml/min/1.73 m^2), suggesting that an imbalance in blood calcium-phosphorus levels driven by impaired renal function may be one of the main potential mechanisms leading to the formation of medial calcification. In summary, chapters 3 demonstrate that intimal and medial calcification are two distinct entities in terms of risk factors and mechanisms of formation, and that understanding the relationship between renal impairment and SPC and IAC patterns may help optimize the prevention and treatment of cerebrovascular disease. In chapter 4, based on our established method using a novel original time and distance assessment technique to directly evaluate human carotid-cerebral pulse wave velocity (ccPWV), for the first time in the literature, revealed that the degree of cerebral arterial calcification was correlated with cerebral arterial stiffness in patients with acute ischemic stroke. In addition, we demonstrated that arterial stiffness as determined by brachial-ankle pulse wave velocity (baPWV) was also positively associated with the degree of IAC in patients with acute ischemic stroke. This is significant as it reflects IAC is not just a particular type of vessel or region phenomenon but reflects the changes of systemic vascular structure and function.

Our research on IAC could greatly advance aging research by clarifying its mechanisms, risk factors, and clinical impacts. Firstly, understanding IAC's pathophysiology may

reveal its role in cerebrovascular diseases and cognitive impairment. Secondly, our findings could spur therapies to dissolve or prevent calcifications, thereby safeguarding brain function in the elderly. Thirdly, linking IAC to cerebral small vessel disease and arterial stiffness may illuminate their combined effects on brain vessel health, paving the way for new strategies to combat cognitive decline and stroke.

In summary, the main goal of studying the metabolic and hemodynamic aspects of IAC is to enhance the prevention and treatment of cerebrovascular diseases, with a particular emphasis on stroke prevention. The first two sub-studies focus on elucidating the risk factors that contribute to the development of IAC. Understanding these causative factors of different pattern calcification is crucial for developing targeted preventive strategies that can be implemented before significant calcification occurs. The latter two sub studies, on the other hand, examine the consequences of IAC. These studies might delve into how calcification affects the structural integrity of blood vessels, contributes to arterial stiffness, ultimately leading to cerebrovascular diseases. The integration of findings from these four sub studies, derived from two cohorts, provides a comprehensive picture of the calcification process and its implications. By viewing these sub studies as interconnected components of a single research trajectory, we can develop a more cohesive strategy for combating cerebrovascular diseases.

5.2 Future Directions

5.2.1 Further longitudinal cohort studies

Firstly, as a cross-sectional study, causal correlation cannot be confirmed, future cohort studies with larger sample sizes are needed. Secondly, since the current research findings were acquired from one-center clinical study, further validations using a public database, or other cohorts will make the findings more reliable. Based on these study findings, our research team will continue this study by exploring comparable public database or collaborating with another research institute for future validations. Moreover, performing a Mendelian randomization analysis may provide an in-depth explanation of the underlying metabolic mechanisms of intracranial artery calcification (IAC). Additionally, the implementation of IAC measures in clinical practice has the potential to improve the diagnosis and management of cerebrovascular diseases such as stroke and vascular dementia. IAC has been shown to be associated with an increased risk of these conditions, and it is of great importance to provides a reliable and objective way to assess the extent of calcification in individual patients. For clinical implementation, there needs to be a standardized method for measuring and reporting IAC. This includes establishing thresholds for what constitutes significant calcification and creating guidelines for interpreting the results. Therefore, further research is needed to establish the clinical utility of IAC measures and to develop user-friendly software tools and standardized imaging protocols. Furthermore, manual IAC measurements are time-consuming and error-prone, hindering research into their etiology and clinical impact, automating IAC assessment using deep learning methods could be a promising

tool for IAC quantification, streamline research and enable clinical use. Finally, compared with high-resolution magnetic resonance imaging, CT is not very sensitive to some atherosclerotic plaques. Therefore, the combination of high-resolution magnetic resonance and CT is very meaningful for many studies.

5.2.2 Cerebral autoregulation, a potential mediating factor between intracranial arterial calcification and cerebral small vessel disease?

Although IAC is most seen in large intracranial vessels, studies have shown a close correlation with cerebral small vessel disease (CSVD). However, its underlying mechanism has not been determined.

Intimal calcification is more related to focal atherosclerotic lesions, whereas medial calcification is associated with increased pulse pressure and arterial stiffness. These physiological changes could potentially impair cerebral autoregulation, a critical mechanism for maintaining consistent cerebral blood flow. There are numerous methods currently available for evaluating cerebral autoregulation, such as transfer function analysis (TFA), autoregulation index (ARI), and the pulpability index (PI).[43,142-144] Over recent years, the measurement of critical closing pressure (CCP) using Transcranial Doppler (TCD) has emerged as a significant method for quantifying CA function.[145,146] CCP, a theoretical pressure threshold below which blood vessels are presumed to collapse and cerebral blood flow approaches zero, has been used in several dynamic cerebral autoregulation models.[147,148] Moreover, CCP, acting as an indicator of cerebrovascular tension, has been identified as an independent predictor of CSVD

burden.[149] Considering the effects of IAC on both cerebral autoregulation and CSVD, we hypothesized that CCP might mediate the association between IAC and CSVD. Thus, in our future studies, we will test the hypothesis that cerebral autoregulation mediates the association between IAC and CSVD burden. We will further investigate the mediation of CCP in different IAC patterns to identify potential mechanisms explaining how IAC might lead to CSVD.

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