VALIDATION OF CT-IDENTIFIED INTRACRANIAL ARTERIAL CALCIFICATION AS A NOVEL IMAGING BIOMARKER FOR REPERFUSION THERAPY OF ISCHEMIC STROKE PATIENTS: SERIAL HOSPITAL-BASED CLINICAL RESEARCH

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Validation of CT-identified Intracranial Arterial Calcification as a Novel Imaging Biomarker for Reperfusion Therapy of Ischemic Stroke Patients: Serial Hospital-based Clinical Research

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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CERTIFICATE OF ORIGINALITY

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_____ (Signed)

Heng Du (Name of student)

Abstract

Background and purpose

Intracranial arterial calcification (IAC) is an independent risk factor for ischemic stroke. For the past years, IAC has been regarded as a proxy of intracranial atherosclerosis based on the high frequency of IAC detection in atheromatous lesions. However, despite the correlations identified between stroke occurrence and IAC, there are still controversies about the connections between IAC and stroke, such as specific cerebrovascular disorder and detailed mechanisms. Serial autopsies showed that there are mainly two patterns of IAC: intimal IAC that involves the intima and medial IAC that is present within the media of the vessel wall. Intimal IAC more frequently coexist with progressive atherosclerosis while medial IAC is associated with arterial stiffness. The difference in pathology may lead to diverse consequences in imaging manifestation, plaque vulnerability and the outcome of treatment. In this thesis, we aimed to delineate the associations between cerebrovascular diseases and different patterns of IAC (intimal and medial) by serial multimodal imaging-based studies.

Methods and Materials

This hospital-based research consecutively recruited patients with acute ischemic stroke or transient ischemic attack who were scanned by brain computed tomography (CT), magnetic resonance imaging (MRI), magnetic resonance angiography (MRA) and high-resolution MRI (HR-MRI). Brain CT was used to examine the presence, patterns and severity of IAC (intimal IAC and medial IAC). MRI was used to evaluate the presence of white matter hyperintensities (WMH) and the infarct pattern in ischemic stroke patients. MRA and HR-MRI

were used to assess arteriosclerotic lesions using detailed imaging features including luminal stenosis, eccentric plaque, plaque burden and intraplaque hemorrhage (IPH). The association between IAC pattern and reperfusion treatment was assessed based on the recovery in neurological function in the acute stage of ischemic stroke. For patients who had reperfusion therapy, neurological dysfunction before and after the treatment was assessed by the National Institute of Health stroke scale (NIHSS). Clinical outcome including favorable neurologic outcome (FNO) and early neurologic deterioration (END) were assessed within 10 days after reperfusion treatment. The etiology of ischemic stroke was assessed based on TOAST classification.

Results

To investigate the correlations between IAC pattern and intracranial large artery arteriosclerosis, we examined 460 intracranial artery segments from 69 consecutively included stroke patients aged between 18 and 80 years old. Bilateral intracranial internal carotid arteries (ICA), middle cerebral arteries (MCA), intracranial vertebral arteries (VA) and the basilar artery (BA) in each patient were screened using non-contrast brain computed tomography (CT) and high-resolution magnetic resonance imaging (HR-MRI). The results showed that intimal IAC was frequently detected in the cavernous (34.3%) and supraclinoid-to-ophthalmic (30.3%) segments of ICA and the V4 segment of the VA (24.2%), while medial IAC was more prevalently present in the cavernous (43.5%) and supraclinoid-to-ophthalmic (40.3%) segment of the ICA. After comparing atherosclerotic lesions assessed by HR-MRI, the results showed that artery plaques with intimal IAC more frequently coexisted with luminal stenosis (p=0.003), eccentric plaque (p=0.02), higher plaque burden (p=0.001) and IPH (p=0.001), compared with medial IAC and non-calcified artery segments. Medial IAC

was not associated with these imaging features of large artery arteriosclerosis.

Analysis on the correlations between WMH and IAC pattern showed different results. We assessed WMH with a detailed eight-grade criteria and categorized the severity of WMH into mild WMH, moderate WMH and sever WMH. To further investigate the correlations between IAC and WMH, we also categorized IAC into diffuse IAC and focal IAC based on its involvement in the affected artery segment. In total, 265 patients were included. Intimal IAC was in 54.7% patients and medial IAC in 48.5% patients. Diffuse IAC was in 74 patients, which were all medial pattern. The analysis showed that medial IAC was independently correlated with the presence of WMH (p<0.001). Moreover, after categorizing the severity of WMH, it was found that patients with more severe WMH were more prone to have higher amount of artery segments affected by medial IAC (p<0.001) and higher involvement (diffuse IAC) of medial IAC in individual artery segment (p<0.001).

For the correlation between IAC pattern and reperfusion therapy, a total of 130 patients who had either intravenous thrombolysis (IVT) or IVT plus endovascular thrombectomy (EVT) were included. Diffuse IAC was associated with higher baseline NIHSS (p=0.011) and less frequent FNO (p=0.047). Compared with patients with focal or single diffuse IAC, patients with multiple diffuse IAC had higher baseline NIHSS (p=0.002) and less FNO (p=0.024). No significant association was found between END and different IAC patterns.

As for the correlations between IAC pattern and the etiology of ischemic stroke, we mainly focused on three most common etiologies: large artery atherosclerosis, small vessel occlusion and cardioembolism. A total of 279 acute ischemic stroke patients were included, with 52.0% having small vessel occlusion, 41.2% having large artery atherosclerosis and 6.8% being identified to have cardioembolism. Statistical analysis suggested no significant

difference in stroke etiology between patients with intimal IAC and patients with medial IAC.

Conclusions

The associations with cerebrovascular disease between intimal IAC and medial IAC are different. Intimal IAC is more correlated with large artery arteriosclerosis and is associated with features that may indicate higher risk of ischemic stroke. On the contrary, medial IAC is more correlated with the presence and severity of cerebral small vessel disease. By affecting cerebrovascular beds differently, intimal IAC and medial IAC may have different effects on reperfusion therapy: patient who were more widely involved by medial IAC tended to have more severe neurological dysfunction and less favorable outcome in the acute stage of ischemic stroke. Since the formation of IAC is more of a chronic process of systematic calcification, the impact of IAC pattern on general stroke etiology may be less significant. Future studies on more sophisticated stroke mechanism such as hypoperfusion, artery-to-artery embolism and penetrating artery occlusion may be needed.

Publications and Presentations

Publication

1. **Du H**, Yang WJ, and Chen XY, Histology-Verified Intracranial Artery Calcification and Its Clinical Relevance with Cerebrovascular Disease. Frontiers in Neurology. 2022 Jan 24;12:789035.
2. **Du H**, Li J, Yang WJ, Bos D, Zheng L, Wong KS, Leung TW, and Chen XY, Intracranial Arterial Calcification and Intracranial Atherosclerosis: Close but Different. Frontiers in Neurology. 2022 Feb 8;13:799429.
3. **Du H**, Zheng JR, Li XL, Bos D, Yang WJ, Cheng YJ, Liu C, Wong KS, Hu J and Chen XY, The Correlation between Intracranial Arterial Calcification and the Outcome of Reperfusion Therapy. Annals of Clinical and Translational Neurology. 2023. doi: 10.1002/acn3.51780.
4. Li XL, **Du H**, Yang WJ, Chen JR, Li XL, and Chen XY, The association of renal impairment with different patterns of intracranial arterial calcification: Intimal and medial calcification. Atherosclerosis. 2022 Dec;363:42-47.
5. Shao HL, Chan WC, **Du H**, Chen XY, Ma QL, and Shao ZY. A new machine learning algorithm with high interpretability for improving the safety and efficiency of thrombolysis for stroke patients: A hospital-based pilot study. Digital Health. 2023 Jan 3;9:20552076221149528.
6. Song XY, Li S, **Du H**, Hu QM, Zhou L, Zhao JL, Gu Y, Hu YM, Lu HY, Wang GD, Chen XY, and Wang QS. Association of Plaque Morphology with Stroke Mechanism in Patients with Symptomatic Posterior Circulation ICAD. Neurology. 2022 Oct 11;99(24):e2708-e2717.

7. Shao HL, Chen XY, Ma QL, Shao ZY, **Du H**, and Chan WC. The Feasibility and Accuracy of Machine Learning in Improving Safety and Efficiency of Thrombolysis for Stroke Patients: Literature Review and Proposed Improvements. Frontiers in Neurology. 2022 Oct 20;13:934929.

Manuscript in submission process

1. **Du H**, Zheng JR, Li XL, Dong YJ, Cheng YJ, Liu C, Hu J, and Chen XY. The Correlation between Medial Pattern of Intracranial Arterial Calcification and White Matter Hyperintensities.

Abstract Presentation

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2. **Du H,** Li XL, Chen XY. Intracranial arterial calcification pattern is associated with early clinical outcome of reperfusion therapy in ischemic stroke. *International Conference on IntraCranial AtheroSclerosis (ICAS Rotterdam 2022). Virtual Conference, oral presentation (16-17 September, 2022)*.
3. **Du H**, Gu Y, Wang QS, Chen XY. Morphological features of intracranial atherosclerotic disease are associated with the recurrence of ischemic stroke. 9th *European Stroke Organisation Conference (ESOC 2023). Poster presentation (Munich, 24-26 May, 2023)*

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List of Abbreviations

CT, computed tomography

MRI, magnetic resonance imaging

HR-MRI, high resolution magnetic resonance imaging

VWMRI, vessel-wall magnetic resonance imaging

TOF-MRA, time-of-flight magnetic angiography

CTA, computed tomographic angiography

IAC, intracranial arterial calcification

ICAD, intracranial atherosclerotic disease

IPH, intraplaque hemorrhage

CSVD, cerebral small vessel disease

WMH, white matter hyperintensities

IVT, intravenous thrombolysis

EVT, endovascular thrombectomy

NIHSS, National Institutes of Health Stroke Scale

FNO, favorable neurologic outcome

END, early neurologic deterioration

SVO, small vessel occlusion

LAA, large artery atherosclerosis

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Chapter 1. General Introduction

Chapter 1. General Information

1.1 Background

1.1.1 Intracranial arterial calcification

Ischemic stroke is a leading cause of death and long-term disability worldwide[1-4]. Among all determined etiologies of ischemic stroke, intracranial atherosclerotic disease (ICAD) is one of the most commonly identified during clinical practice, which accounts for multiple mechanisms of stroke[5]. For the past years, intracranial arterial calcification (IAC) has been regarded as a marker of ICAD based on its high concurrence in atheromatous lesions[6,7]. However, recent histopathological evidence revealed discrepancies between IAC and ICAD: In large intracranial arteries, substantial calcifications exist in both intimal (inner) layer and medial (middle) layer of the arterial wall[8,9] while atherosclerosis is a series of pathological processes that mainly involve the intimal layer[10]. Two latest autopsies on intracranial arteries had demonstrated a high percentage of medial calcifications (62% and 87%, respectively) with an absence of ICAD[8,9]. Although detailed mechanisms of the formation of medial calcification has not been fully revealed yet, it seems less associated with the process of atherosclerosis compared to that of intimal calcification. Despite IAC as an independent risk factor for ischemic stroke[11,12], the pathophysiology of IAC-related cerebrovascular diseases has not been fully studied, either. By far, controversies still exist about IAC in several aspects, such as the association with territorial cerebral infarction[13-15] and the prognosis evaluation of ischemic stroke[16-18]. In consideration of the inherent pathological characteristics of specific patterns of IAC, further studies are needed on their latent difference, including mechanism analysis, prognosis evaluation and so on.

Computed tomography (CT) is a widely applied imaging technique in detecting the presence of IAC. In previous studies, the severity of IAC was mostly evaluated by visual grading scale according to the involved circumference of the vessel wall and the thickness of

the calcified area[19,20]. However, such method does not include any differentiations of IAC pattern, which may lead to potential controversies in many aspects. Recently, an advanced grading system was developed. It provided classifications of IAC pattern based on the cross validation on the imaging and histological feature of IAC[21]. The optimized IAC scoring system enables further investigations to unravel the contradictory status of the association between IAC and ischemic stroke, including stroke mechanism, cerebral vascular collateral circulation, prognosis evaluation and so on.

1.1.2 Reperfusion therapy in ischemic stroke

During ischemia, oxygen is insufficient to support triphosphate (ATP) and the activity of neurons fails rapidly in minutes[22]. Due to the susceptibility of brain to hypoxia-ischemia, the importance and urgency of reperfusion of ischemic stroke have always been a major concern. In 1995, a breakthrough was made in reperfusion therapy with the application of intravenous recombinant tissue plasminogen activator (rt-PA, alteplase)[23]. Alteplase is a glycoprotein. Once combined with fibrin, it will be activated for the conversion of plasmin, which leads to fibrin degradation and blood clot dissolution[24]. The therapeutic time window of alteplase is 3-4.5 hours[23,25]. Despite the benefit in preventing post-stroke disability, it has limitations: a narrow time window, an increased risk of large parenchymal hemorrhages[26-28] and a low recanalization rate of large occluded arteries[29,30]. Validated in 2015 by five randomized clinical trials[31-35], endovascular thrombectomy (EVT) has been taking the dominant role in large artery occlusions with an expansion of time window to 6 hours. By pulling the thrombus out of the vessel with stent retrievers, EVT would consequently benefit more in revascularization, disability prevention and death reduction[36].

Hemodynamics status of stroke patients always draw the interest of neurologists during

clinical practice[37,38]. In reperfusion therapy, the status of collateral circulation and perfusion are two of the dominant factors that influence the rate of successful reperfusion and outcome[39,40]. On the other hand, impaired distal perfusion status or blood flow may act as a risk factor of recurrent ischemic stroke[37]. In consideration of the correlation of IAC and ischemic stroke, studies on the impact of IAC on collateral and perfusion status are needed.

1.2 Objectives and organization of the thesis

1.2.1 Objectives

Figure 1-1 illustrates the process of subject inclusion and analysis of this study. This hospital-based study aimed to explore and validate CT-identified IAC pattern as a novel imaging biomarker for cerebrovascular disease and optimizing reperfusion therapy of individual stroke patients. It consisted of four major objectives:

1) To investigate the potential correlation between CT-identified IAC and vessel wall MRI-identified large artery disease (intracranial atherosclerosis) among stroke patients;

2) To investigate the association between CT-identified IAC and MRI-identified small vessel disease (white matter hyperintensities) among stroke patients;

3) To investigate the potential effects of IAC on the severity of ischemic stroke and the efficacy of reperfusion therapy, which will be evaluated in the aspect of severity, functional improvement, early neurological deterioration;

4) To examine the correlation of IAC with acute stroke etiology, especially lacunar infarct.

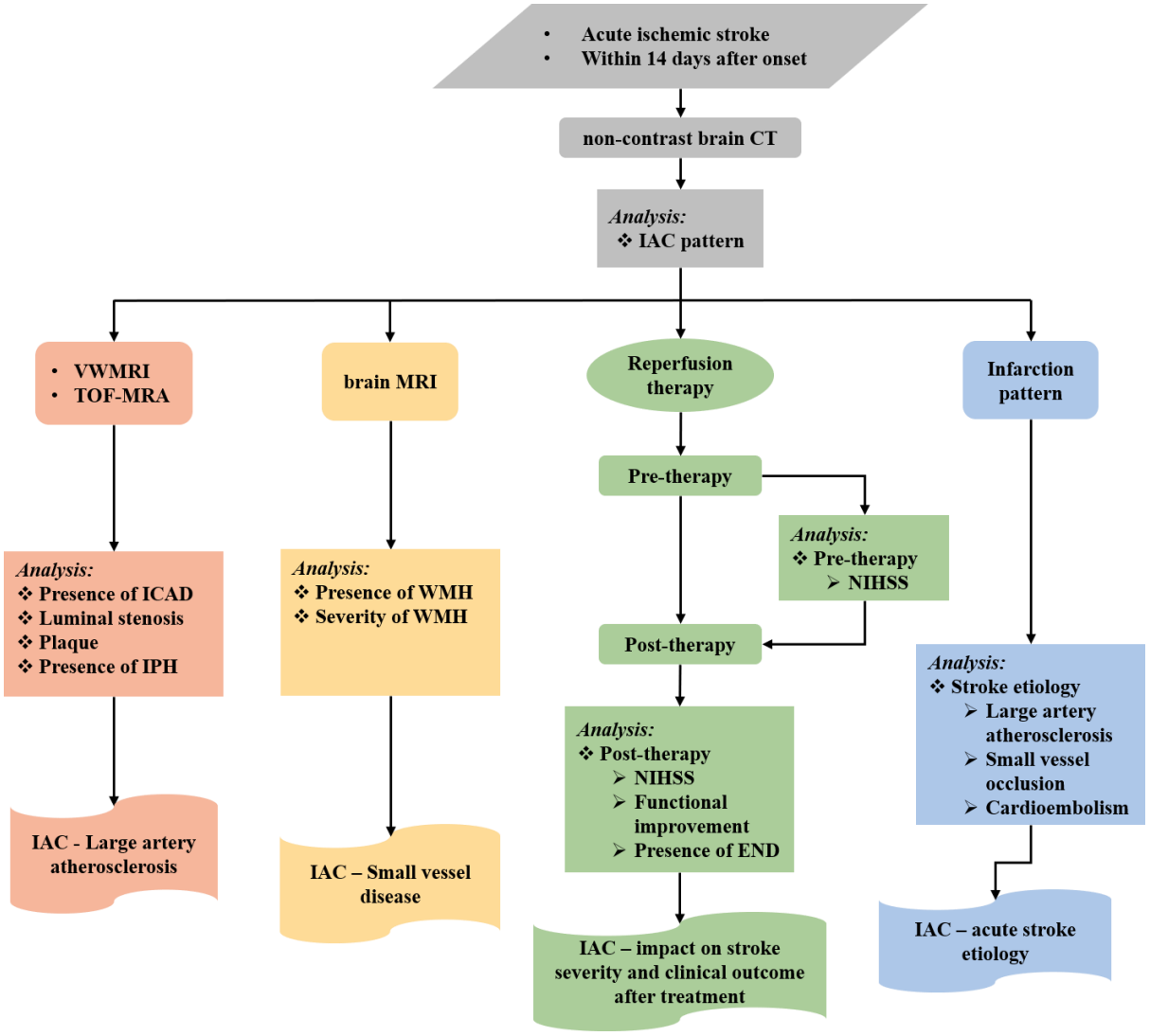

Figure 1-1. The process of this hospital-based study. CT, computed tomography; **IAC**, intracranial arterial calcification; **MRI**, magnetic resonance imaging; **VWMRI**, vessel-wall magnetic resonance imaging; **TOF-MRA**, time-of-flight magnetic angiography; **ICAD**, intracranial atherosclerotic disease; **IPH**, intraplaque hemorrhage; **WMH**, white matter hyperintensities; **NIHSS**, National Institutes of Health Stroke Scale; **END**, early neurologic deterioration.

1.2.2 The organization of the thesis

This study is divided into seven 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, in which IAC is systematically investigated based on the differentiation of IAC pattern. In Chapter 2, intimal IAC and medial IAC are compared according to the aspect of imaging evaluation, histopathological characteristics, prevalence and distribution and clinical relevance. Chapter 3 focuses on the correlation between IAC and large artery disease. It presents a cross validation of IAC and ICAD by brain CT and VWMRI, which explores the difference in morphological features between intimal IAC and medial IAC. Chapter 4 focuses on the correlation between IAC and small vessel disease. The presence and the severity of WMH was investigated in stroke patients with different patterns and burdens of IAC. In Chapter 5, the correlation between IAC pattern and reperfusion therapy is studied. Risk factors, the severity of stroke, reaction to reperfusion therapy and clinical outcomes were analyzed based on different patterns and burdens of IAC. The correlation between IAC pattern and acute stroke etiology is described in Chapter 6, which mainly focused on lacunar infarct. Chapter 7 is conclusions of this theses and perspectives for future studies.

Chapter 2. Literature Review: Histology-verified Intracranial Arterial Calcification and Its Clinical Relevance with Cerebrovascular Disease

Chapter 2. Literature Review

2.1 Introduction

Calcification is widely located in all vascular beds[41,42], especially in the advanced stages of atherosclerosis along with intraplaque hemorrhage, hemosiderin deposition and lumen surface disruption[43]. Over the last decades, significant progress has been made in clinical research on intracranial arterial calcification (IAC). IAC in the intracranial internal carotid artery (ICA) was demonstrated as an independent risk factor for ischemic stroke which accounted for up to 75% of all strokes[11]. Currently, most studies on IAC are based on computer tomography (CT) which is capable of providing overall views of single or multiple calcifications. Calcification score and volume[44], which were initially used for assessing coronary arteries, are now widely applied into qualitative and quantitative measurements in exploring the clinical relevance of IAC[45]. Despite substantial research on IAC, the correlation of IAC with stroke is controversial. In the Rotterdam study, many of the ischemic strokes were either in the vascular territories that were separate from IAC or caused by other conditions, for instance, cardiac source embolism or coexisting penetrating artery diseases[11]. Furthermore, despite the association of IAC and intraplaque hemorrhage[46], calcified atherosclerotic plaques in the middle cerebral arteries seemed to be more stable than non-calcified plaques[13]. The pathophysiology of IAC and stroke remains unclear.

There are two major patterns of IAC, one involving the intima and the other involving the media. Recent histopathologic evidence showed that medial calcification was predominantly present in the intracranial segments of internal carotid arteries (ICAs)[9] and vertebral arteries (VAs)[8], bringing about new considerations on the importance of IAC patterns in further clinical studies. In this review, we intended to discuss the histopathological features of IAC, its manifestation in computed tomography (CT) and magnetic resonance imaging (MRI) as well as its clinical relevance, which may benefit in developing a better understanding of calcification and related diseases.

2.2 Methods

Literature searching was performed in PubMed, with a search filter using words, such as “intracranial arterial calcification”, “intimal calcification”, “medial calcification” and “stroke”. All articles were extracted according to title and abstract. Most of the articles were original clinical studies. The full texts of the relevant articles were assessed independently. The references of the relevant articles were selected additionally for further evidence. Three hundred and six-eight articles about either the intracranial artery or the extracranial artery were screened. Articles based on autopsies, CT and MRI were included. Articles focused on biochemical or genetic studies were excluded.

For studies based on autopsies, the identification of intimal or medial calcification in the intracranial artery was based on pathological evidence. Calcifications were identified as sharply demarcated, acellular spots and areas. Calcification type was determined by adding the calcification areas in all slides to a summed intimal and medial calcification burden. If the summed area of medial calcification was larger than the summed area of intimal calcification, the patient was categorized by histology as medial dominant and vice versa.

For studies based on brain CT, the definition of intimal or medial calcification in the intracranial artery was based on circularity (1 for dot, 2 for <90°, 3 for 90-270° and 4 for 270-360°), thickness (1 for thick IAC ≥ 1.5mm and 3 for thin IAC < 1.5mm) and morphology (0 for indistinguishable, 1 for irregular/patchy and 4 for continuous) on brain CT. A summed score from 1 to 6 indicated predominantly intimal calcification and 7 to 11 indicated predominantly medial calcification. For studies based on MRI, the identification of IAC pattern is yet to be studied.

2.3 Imaging measurement on IAC and the existing defects

Intracranial arterial calcification is widely detected by brain CT scan due to its accessibility and reliability[47]. In order to achieve quantitative analysis, semiautomatic custom-made methods are used *via* software, such as ImageJ[48] or MATLAB[14]. The "volume" of IAC is calculated by multiplying the number, size and the increment of pixels. Agatston score[49], which represents the area of calcified plaque multiplied by weighted value assigned to its highest Hounsfield unit, has also been applied in the studies. Deficiently, Agatston score and calcium volume are both in demand for the slice thickness of 3 mm and for images without gantry tilts[45] and, moreover, both are time-consuming and sometimes inaccessible for neurologists during clinical practice. In contrast, visual grading systems are comparatively more convenient for assessments. According to the previous grading criteria, the score increases as calcification extends either in the thickness or in the circumference[19,20,50].

However, since IAC embodied in intima and media of intracranial arteries can vary in morphology and prevalence, the misleading effect of Agaston score or overall volume should not be neglected. In 2017, a new grading system was put forward by Kockelkoren, et al.[21] which distinguished the intimal and medial calcification after comparisons of histology and CT features of IAC. Circularity, thickness and morphology were counted separately (**Table 2-1**) based on the distinct features of intimal and medial calcification. Of note, the grading order of thickness was conversed ("thick" represents one point and "thin" represents three points) and morphology was added into counting compared to prior grading systems[51].

One of the shortages of the visual systems is the subjectivity between distinct grading scales, which is the notable obstacle in maintaining consistencies. Another considerable defect is the interference by adjacent bony structures, for instance, the skull base around the carotid siphon and the VA[45]. Recently, high-resolution MRI (HR-MRI) has been applied to evaluate

lesions of intracranial vessel walls[52]. By presenting hypointensity, IAC can be detected by HR-MRI. Revealing the ultrastructure of the vessel wall, HR-MRI enables the neurologists to identify the calcium deposits at different locations (e.g. superficial, deep) and their positional relationship with other plaque components[53]. By comparing the autopsy and multi-contrast HR-MRI, Jiang, et al.[54] accomplished a remarkable differentiation between lipid core (isointense/hyperintense), fibrous cap (isointense) and calcification (hypointense) on T1 sequence. The combination of HR-MRI and CT will presumably benefit both diagnosis and differentiation of IAC and, in addition, will indicate a necessity of classification of IAC located at different layers of the vessel wall (intima and media).

Table 2-1. The grading scale (by Kockelkoren, et al[21]) for differentiation between intimal calcification and medial calcification.

Characteristic: **circularity, thickness and morphology**		Points
Circularity	Absent	0
	Dot(s)	1
	<90 degrees	2
	90-270 degrees	3
	270-360 degrees	4
Thickness	Absent	0
	Thick ≥ 1.5mm	1
	Thin < 1.5mm	3
Morphology	Indistinguishable	0
	Irregular/Patchy	1
	Continuous	4
< 7: Dominant Intimal IAC; ≥ 7: Dominant Medial IAC		

2.4 Histological features of calcifications

The structure of the wall of intracranial arteries consists of three layers: the intima (the inner layer), the media (the middle layer) and the adventitia (the outer layer). Different from extracranial arteries which are rich in elastin filaments, intracranial arteries own characteristic features with a denser internal elastic lamina, a thinner media with few elastic fibers, a less abundant adventitia and an absence of external elastic lamina[55]. Vascular calcification, resembling osteogenesis, reflects an osteochondrogenic transformation of smooth muscle cells. Traditionally, calcification is deemed to imply atherosclerosis. Studies in the early 20th century conducted by Mönckeberg confirmed that the origination of medial calcification was independent of atherosclerosis[56]. Due to differed histological features, calcifications in the intima and the media ought to be discussed separately.

2.4.1 Intimal calcification

Intimal calcification is characterized by subintimal lipid deposition and macrophage accumulation[57]. The intimal layer comprises endothelial cells and subendothelial connective tissue. During atherosclerosis, inflammation intrudes and the intima becomes thickened gradually with the formation of calcification[58]. While absent in primary types, calcium deposits begin to occur as atherosclerosis advances. **Figure 2-1** shows the formation and distribution of intimal calcification. Initially appearing as granules within or outside the injured smooth muscle cells, calcifications diffusely scatter among extracellular materials and some of which are internalized by macrophage foam cells. With a continuous fusion of adjacent granules, calcified granules will turn into larger clusters containing lumps and plates of calcium[56]. In some cases, large calcifications may ulcerate the intima, leading to subsequent occlusions[59].

Intimal calcifications frequently occur as thick and patchy clusters[21,60]. Similar to

extracranial arteries, histological evidence indicates that intimal calcification frequently (85%[9] and 100%[8]) coexists with intracranial atherosclerotic disease (ICAD), but its prevalence among all-stage ICAD lesions is not comparatively high (62%[9] and 69%[8]). This discrepancy could be attributed to the presence of intimal calcification, which is mainly in the progressive ICAD lesions instead of pre-ICAD lesions[8].

Vasa vasorum is a microvasculature network in vessel walls which delivers oxygen and nutrition[61] and transports inflammatory mediators[62-64] that could contribute to atherosclerosis. In 2018, Zheng, et al.[65] firstly reported the association between the density of intraplaque calcification and the presence of adventitial vasa vasorum in *ex vivo* VA specimens, indicating a mutual basis of calcification and ICAD. However, whether intimal or medial calcification is associated with vasa vasorum is yet unknown.

2.4.2 Medial calcification

The medial layer of the intracranial vessel wall consists of smooth muscle cells and elastin-rich extracellular matrix. The normal thickness of the media in the middle cerebral artery (MCA), basilar artery (BA) and VA ranges from 0.15mm to 0.19mm, and it tends to decrease during the pathological changes such as atherosclerosis[43]. Medial calcifications are deposits of hydroxyapatite with a high degree of crystallization[66]. In **Figure 2-1** the formation and extension of medial calcification is briefly drawn. A four-stage criterion is applied to distinguish the calcified lesion in the medial layer[67]: 1) irregular distribution of intracellular deposits in vascular muscle cells and extracellular deposits near damaged elastic fibres in the media (colored in blue or violet) on H&E staining; 2) confluent calcification extending up to incomplete circumference with subendothelial hyperplasia in the intima ; 3) calcifications distorting the architecture of media and involving the entire circumference; 4) calcification foci

of bone formation.

Focal inflammation also plays a role in medial calcification. A high level of inflammatory markers like C-reactive protein, CD40, and CD154 can be identified in the vicinity of calcified media[68]. The anomalous expression of mineral-regulating proteins may contribute to the process. In patients with chronic kidney disease (CKD), rapid development of medial calcification in the extracranial arteries is observed, partly due to mineral dysregulation stemming from the primary renal disorder[67]. Endoplasmic reticulum stress[69] and inflammasome activation[70] may have an impact on the medial calcification, but the key pathogenesis remains hidden.

Medial calcifications appear as thin, continuous and circular lesions[21,60]. The formation of medial calcification is thought to be independent of atherosclerosis. In intracranial ICAs, the earliest calcifications are located in the medial layer and are unrelated to ICAD[71]. More than 60% of non-atherosclerotic medial calcifications in intracranial ICAs which extend over half of the circumference are irrelevant to the occurrence of the intimal calcification or ICAD while the prevalence of concurrent calcifications is merely 9%[9].

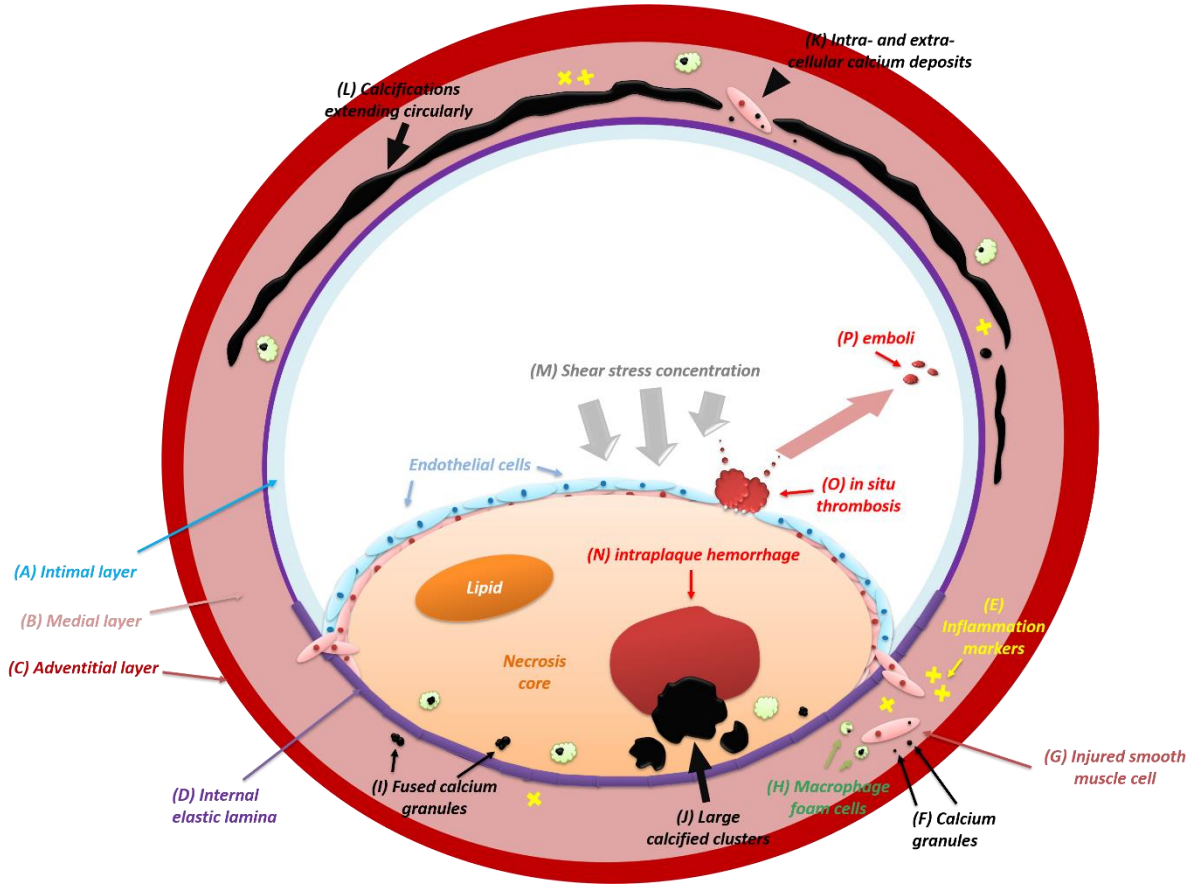

Figure 2-1. The formation and distribution of two patterns of intracranial arterial calcification (intimal calcification and medial calcification). IAC mainly involves the intimal layer (A) and the medial layer (B) instead of the adventitial layer (C). During the initial process of atherosclerosis, inflammation (E) infiltrates constantly and intimal calcifications begin to emerge. Usually, calcifications appear as calcium granules (F) within or outside the injured smooth muscle cells (G) and scatter diffusely among extracellular materials. Some of the calcium deposits will be internalized by macrophage foam cells (H). After growing in size with continuous fusion (I) of adjacent granules, calcified deposits will turn into large structures (J). Eventually, with the intima (A) ulcerated by large calcifications, in situ thrombosis (O) will adhere to the bare surface of the lesion and release emboli (P) subsequently. In some cases, the shear stress upon the arterial wall will be concentrated (M) on the lesion where large intimal calcifications are present. The elevated shear stress will result in the deformation and rupture

of neovessels inside the lesion, leading to intraplaque hemorrhage (N). At first, medial calcifications are irregular calcium deposits (K) distributed intra-cellularly in the vascular muscle cells (G) and extra-cellularly near damaged elastic fibers (D) in the medial layer (B). After confluent extending (L) up to incomplete circumference, medial calcification will distort the architecture of the medial layer and then involve the entire circumference of the vessel wall.

2.5 The prevalence and distribution of IAC

The highest prevalence of IAC is found in intracranial ICA (60-80%), followed by that in intracranial VA (17-35%), compared to BA (2.5-7%) and MCA (5%)[12,72,73]. On histology, a low prevalence (3%) of IAC in major intracranial arteries of Caucasians was reported[74]. However, the prevalence of IAC in that of Chinses adults was higher (27.9% in MCA[75] and 39% in the major intracranial arteries[8]).

Different intracranial arteries may have diverse frequency of IAC. Homburg, et al.[76] reported a low prevalence of calcified ICAD lesions (23%) in distal branches of the circle of Willis. Comparatively, the cavernous and carotid siphon are the most common sites of calcification in the ICAs due to their tortuous anatomical configuration[19,77]. Among all segments of VA, the intracranial part is most frequently affected by calcification[72]. Left intracranial VA is found more frequently calcified than the right and most of the IACs in VAs are focal lesions[78], but the mechanism of the left-and-right difference is unclear.

While intimal calcifications tend to occur in all major intracranial arteries (ICA, MCA, VA and BA) and are always concurrent with ICAD[8,9], medial calcification is more predominantly present in the ICA[9] and the VA[8]. Histological findings demonstrated that medial calcification contributed more to the total calcified cross-sectional surface area of the carotid artery than intimal calcification (79% vs. 14%)[9], which indicates their difference in imaging feature (cluster vs. circular). However, it was also identified that 36% of the medial calcification had a maximally affected circumference <50%, meaning a potential cluster-like pattern[9]. As a result, the overall calcification on CT scan might not serve as accurate proxy of atherosclerosis.

2.6 Clinical relevance of IAC

2.6.1 Risk factors

Age[79-81] is an independent risk factor for IAC. In 2005, a CT-based study consisting of 490 consecutive cases demonstrated a high prevalence of calcification (69.4%), among which patients with IAC were significantly older[72]. Diabetes[82-84] and chronic kidney disease[85,86] are two other major risk factors. As for gender difference, women are found with milder calcification than men[48,87], but there is a contradictory finding, too[88]. Opposite findings were also reported about hypertension[89,90]. One hypothesis suggests that IAC-related arterial stiffening may act as a cause for the elevated pulse pressure.

Although intimal calcification and medial calcification share mutual risk factors, such as age and higher pulse pressure[71,91,92], there are some difference in other traditional risk factors (**Table 2-2**). After differentiating into different IAC patterns (intimal and medial), male gender and smoking were found to be independently related to intimal calcification, while aging and diabetes mellitus were more related to medial calcification[91,93].

Table 2-2. Comparison of risk factors between intimal and medial calcification (intracranial arteries and periphery arteries).

Study authors and year	Subjects	Sample size	Risk factors	
			Intimal calcification	Medial calcification
Vos et al., 2018[91]	Intracranial ICAs of patients with acute ischemic stroke who received CT scans	1132	Male, smoking, hypertension,	Age, diabetes mellitus, previous vascular disease
Compagne et al., 2018[16]	Intracranial ICAs of patients who received CT scans and underwent thrombectomy	344	Male, smoking, pre-stroke with mRS ≤ 2	Age, atrial fibrillation, diabetes mellitus, myocardial infarction, hypertension
Golüke et al., 2020[92]	Intracranial ICAs of patients with mixed dementia who received CT scans	1992	Male, hypertension, smoking, myocardial infarction	Diabetes mellitus
Zwakenberg et al., 2020[94]	Femoral arteries of patients who received CT scans	718	Smoking, history of peripheral arterial disease, higher osteonectin level	Age, diabetes, HbA1c, higher ankle brachial index (ABI), higher dp-ucMGP level

2.6.2 Plaque vulnerability

The association of IAC with plaque vulnerability remains controversial, which is partially due to the unclassified calcification patterns. A CT study showed that asymptomatic MCA plaques had a higher frequency of IAC compared to symptomatic MCA plaques[13], implying that IAC has a protective effect on the intracranial arteries. On the other hand, micro embolic signals in the intracranial ICA ipsilateral to acute MCA infarct were more frequently detected by transcranial Doppler (TCD) in patients with a higher extent of calcification (widest arc of IAC $\geqslant 90°$) than those with lower extent of calcification[14]. However, in that TCD-based study[14], patients were diagnosed with concomitant MCA stenosis; hence whether the embolic signals in the temporal window originated exactly from the calcified lesion of ICA was indistinguishable. Moreover, latent carotid calcification is also related to the risk of cardio-aortic embolism[95], which can be a major interference in emboli detection.

So far, knowledge of the plaque vulnerability in different IAC patterns is still limited. In contrast, the link between calcification and plaque vulnerability in the coronary artery and the carotid artery is more thoroughly studied, which may serve as a reference. In coronary atherosclerosis, the superficial calcified nodule is an independent risk factor for plaque rupture[96]. One of the most vulnerable sites is the junction between calcification and soft plaque within the fibrous cap, where the shear stress tends to increase and eventually causes rupture[97]. In the carotid artery, superficial calcification in the plaque was related to intraplaque hemorrhage (IPH)[46,98], a strong predictor for ipsilateral cerebrovascular events[99-101]. One possible explanation about the pathophysiology of calcification related IPH is the change of focal pressure. Due to the presence of calcification, the shear stress of the blood flow is elevated and concentrated on the plaque surface (**Figure 2-2**)[102,103], which may cause the deformation and rupture of neovessels inside the plaque[104]. Compared to superficial calcifications, deep

calcium tends to show irrelevance to IPH in the carotid artery[46]. Since "deep" calcified deposits are more possibly located in the medial (or adventitia) layer, medial calcification appears to be unrelated to plaque vulnerability.

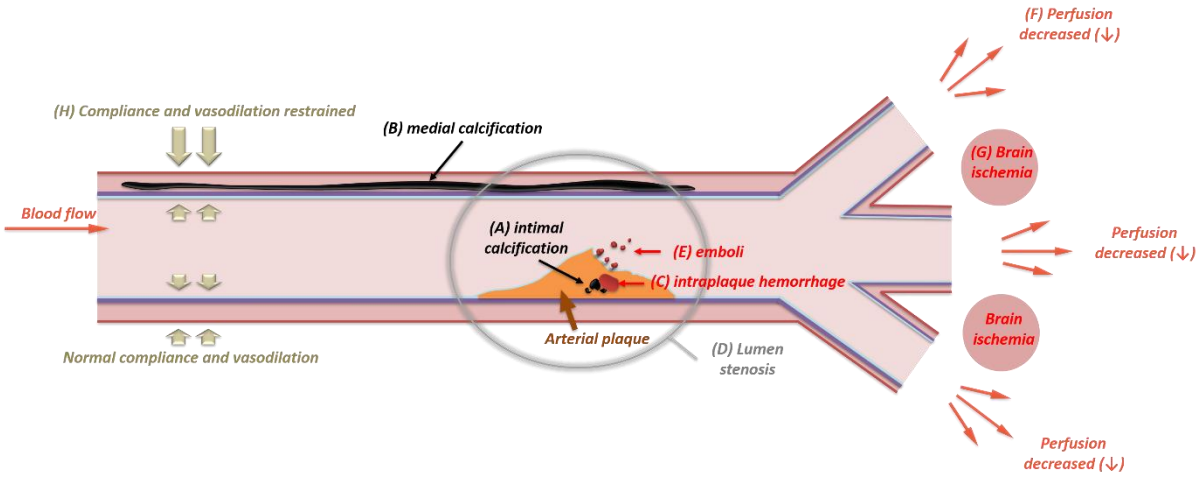

Figure 2-2. The impact of intracranial arterial calcifications (intimal and medial) on ischemic stroke. The presence of intimal calcification (A) may induce intraplaque hemorrhage (C). Intraplaque hemorrhage is one of the major causes for the rapid expanding of arterial plaque, which will then result in luminal stenosis (D). In some cases, the intima will be ulcerated by large calcification, leading to distal embolism (E). With an increase in the stenotic degree, the perfusion of the downward vascular territory will decrease (F), leading to brain ischemia (G) in the border zone of adjacent vascular branches. Unlike intimal calcifications, medial calcification (B) mainly causes arterial stiffness. The stiffened arterial wall will have limited compliance and vasodilation (H), which can be measured through carotid-femoral pulse wave velocity, systolic flow velocity, and pulsatile index.

2.6.3 Hemodynamic impact

Intimal calcification is related to atherosclerotic luminal stenosis[8], a pivotal cause of territorial hypoperfusion and artery-to-artery embolism (**Figure 2-2**)[105]. Previous studies have revealed the correlation between luminal stenosis and the severity (thickness and circularity) of calcification in the intracranial ICA[106,107]. In contrast, medial calcification seems to be more associated with vascular remodelling than luminal narrowing[108] in the VA and coronary artery[109]. No significant correlation between medial calcification and luminal stenosis has been established yet[8]. Medial calcification is thought to affect arterial stiffening, resulting in compliance deterioration and vasodilation limitation (**Figure 2-2**)[110]. However, in previous studies about intracranial arteries, the IAC pattern was seldom categorized. Notwithstanding, the correlation of medial calcification is still partially deducible. Patients with IAC have higher carotid-femoral pulse wave velocity[111]. Additionally, in the MCA and VA, heavier IAC may lead to elevated systolic flow velocity and pulsatile index, which indicate high resistance within the cerebral-vasculature[112]. IAC may also protect the artery from vasospasm under the circumstance of aneurysmal subarachnoid hemorrhage[113], suggesting a restricted vasodilation of affected arteries.

Arterial wall stiffening caused by calcification is an independent risk factor for all-cause mortality[114]. Compagne, et al. reported a trend towards worse outcome in patients with medial calcification who would benefit more after endovascular thrombectomy compared to intimal calcification[16]. Severe calcification is associated with incomplete arterial revascularization after mechanical thrombectomy[18] and prolonged procedural times during endovascular therapy[115]. Besides the luminal stenosis caused by IAC, arterial stiffness is also a detrimental factor in the process of endovascular thrombectomy that blocks the extraction of thrombus and therefore increases the passes of retriever. Furthermore, patients with heavier IAC burden in either anterior[116] or posterior[117] circulation tend to have poor clinical outcomes

after endovascular thrombectomy. It is conceivable that the loss of elasticity may decrease the microvascular cerebral perfusion, resulting in arterial flow stasis and diffuse thrombogenesis. In some diseases, IAC is found protective. Patients with IAC tend to suffer less often from arterial dissection than those without[91]. Whether stiffening acts as a reason is unknown.

2.6.4 IAC in cerebrovascular events

Intracranial arterial calcification has drawn attention as an independent risk factor for stroke[11]. Chen et al.[12] first described a high prevalence of IAC in Chinese adults with ischemic stroke. An upgoing incidence of early vascular events lies toward the severity of IAC in ischemic stroke or transient ischemic attack[118]. Patients with heavier calcification are at higher risk of large cerebral artery occlusion[119] and recurrent ischemic stroke[120,121]. However, despite the fact that ischemic stroke is a condition with divergent causes including large artery atherosclerosis, cardiovascular embolism, small vessel disease and other determined or undetermined etiology, prior studies mostly focused on the association of IAC with all cause of stroke. The Rotterdam study revealed the association of IAC with stroke[11], but many of the stroke events were in the vascular territories that were separate from IAC and were led by other vascular disorders[122]. In a prospective study on patients with ischemic stroke, the presence and score of IAC were found to be associated with recurrent stroke events[17]. However, patients with vascular events had more intracranial atherosclerotic plaques, which may also account for infarction. The coexistence of IAC, atherosclerotic plaque and luminal narrowing make it difficult to be determined. In contrast to these findings, a protective effect of calcification was reported[13], but further studies with a larger sample size and more specific IAC classifications are needed.

Intracranial arterial calcification also has an impact on lacunar infarcts and white matter

hyperintensity[73,123], presumably resulting from an injured vascular tone and vasodilatory after endothelial impairment[124,125]. Erbay et al.[126] reported a weak link of IAC to white matter hyperintensities after adjusting for age. In terms of cerebral hemorrhage, significant expansion of hematoma was observed in the presence of IAC[127]. Evidence showed IAC was a predictor for microbleeds[83,128] and hemorrhagic transformation after intravenous thrombolysis[129,130]. Medial calcification seemed more correlated to hemorrhagic complications after intravenous thrombolytic therapy[131]. The increased frequency of microbleeds in patients with IAC may be due to microvascular impairment[132]. Recently, a possible link was reported between Fahr's disease (familial idiopathic basal ganglia calcification) and calcified small vessels that supply the basal ganglia[133]. It suggests that small vessel impairment may be attributed to IAC as it affects vessel beds systemically.

The pathophysiology of calcification leading to cerebrovascular disease has not been fully elucidated. Intimal calcification often coexists with atherosclerosis, during which endothelial function will be impaired[134] and the permeability of the brain-blood barrier may increase subsequently. Medial calcification can lead to arterial stiffness by damaging the elastic fibre around the internal elastic lamina of the medial layer. With deterioration in compliance, distal cerebral microvascular with increased blood pressure will be vulnerable to rupture.

2.6.5 IAC with cognitive disorder

The association between cognitive disorder and IAC has been studied in recent years. A cross-sectional study with 1992 recruited patients who were diagnosed with different types of cognitive dysfunction[135] revealed a high incidence (about 95%) of intracranial ICA calcification[92]. Cognitive impairment had been observed among patients diagnosed with IAC and concurrent conditions, such as chronic hypoparathyroidism[136] and hemodialysis[137] that

could directly result in irregular serum calcium. Among patients without such conditions, the risk of dementia could also rise with larger IAC volume[138], despite the influence of stroke. Additionally, patients with larger IAC volume[139] or area[140] were found to perform worse during neuropsychological assessments. However, the link between IAC and type of dementia or cognitive disorder turned uncertain after adjustment for age and gender. Similar findings were reported in the severity of mixed dementia, which was identified as "not associated" with IAC either after additional adjustment for cardiovascular risk[92]. The correlation of IAC to cognitive dysfunction remained unclear. One possible cause could be the focus only on IAC in the intracranial ICA, since the prevalence of IAC in other vessel beds is comparatively much lower.

Due to its long preclinical stage in which subtle cognitive deficits could only be revealed by dedicated neuropsychological tests[141], dementia or cognitive decline is always barely noticed by patients until the emergence of evident symptoms. Whether IAC can serve as a bio-marker for early screening of dementia or cognitive decline might depend on further studies including larger number of recruited patients with more intracranial arteries.

2.7 Discussion

Intracranial arterial calcification includes two major types: intimal calcification and medial calcification, in which the histopathological features are different from each other. **Table 2-3** summaries the main difference between intimal calcification and medial calcification. Intimal calcification is more related to focal atherosclerotic lesion while medial calcification tends to spread over the medial layer. Non-atherosclerotic medial calcification is predominantly present in both intracranial ICA and VA while intimal calcification can occur in all major cerebral arteries. Due to different histological features of intimal and medial calcification, the traditional quantitative measurement could be insufficient to reflect on accurate clinical

information, indicating a demand for new measurements by CT or MRI.

Intimal calcification differs from medial calcification in risk factors, the association with plaque vulnerability, and the hemodynamic impact. It plays a critical role in IPH and luminal stenosis while medial calcification is more connected to arterial stiffness and vasodilation. The causal correlation of calcification with infarction and influence of separate IAC patterns are unknown. IAC has been recently found to be a potential risk factor for cerebral small vessel disease, most likely due to the endothelial dysfunction. The pathophysiology underlying the IAC-inducing stroke is still unclear. Further histological, imaging, and clinical evidence that are based on different IAC patterns are required in future studies.

Table 2-3. Summary of the difference between intimal calcification and medial calcification.

IAC pattern	Pathological feature		Risk factor (difference)	Clinical impact	
	Formation	Morphology		Stroke mechanism	Clinical prognosis
Intimal calcification	(1) Related to atherosclerosis, often in advanced stages; (2) Inflammation associated; (3) Granules initially, fuse into large lumps and plates of calcium (4) May ulcerate the intima	Thick / patchy clusters	Male gender; Smoking	(1) Elevated shear stress causing IPH; (2) Hypoperfusion (luminal stenosis)	With lower incidence of hemorrhage after intravenous thrombolysis
Medial calcification	(1) Irrelevant to atherosclerosis; (2) Inflammation associated; (3) Four-stage formation: from deposits to confluent calcification involving the entire circumference	Thin, often in a circular pattern	Aging; Diabetes mellitus; Chronic kidney disease;	(1) Arterial stiffness; (2) Possibly worse cerebral perfusion in microvascular beds	With a trend toward worse outcome which may improve more after endovascular treatment

Chapter 3. Substudy-1: Intracranial Arterial Calcification and Intracranial Atherosclerosis: Close but Different

Chapter 3. Substudy-1

3.1 Background

Intracranial arterial calcification (IAC) is commonly seen in major cerebral arteries[13,78,95] and established as an independent risk factor for stroke[11]. In previous studies, grading by extent and thickness qualitatively[20] and calculating volume with specific software quantitatively[48] were the most frequently applied evaluation methods on IAC. However, both methods lack consideration about the inherent difference between IACs in separate layers of vessel wall, which may act as a potential cause to contradictory findings[13,14].

Intracranial arterial calcification mainly involves the intimal layer and the medial layer, which could vary in histopathological feature and imaging feature [21]. Histopathological studies showed that the intracranial arteries with intimal calcification had more severe lumen stenosis as compared to that without intimal calcification[8,9]. Imaging based studies reported that the medial calcification was associated with poorer collateral status[16] and higher frequency of post-intravenous-thrombolysis hemorrhage[131] compared with intimal calcification. The difference between IAC patterns brought about a need of advanced details about the correlation of IAC with intracranial atherosclerotic disease (ICAD). Therefore, we aimed to compare the morphological features between intracranial plaques with intimal calcification and those with medial calcification.

3.2 Materials and methods

3.2.1 Subjects

The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. This was a retrospective study based on a prior prospective study which was from 2014 to 2018 at the Prince of Wales Hospital, Hong Kong. The inclusion criteria were as follows: 1) patients above 18 years old with acute ischemic stroke 2) brain

computed tomography (CT), routine magnetic resonance imaging (MRI), magnetic resonance angiography (MRA) and vessel-wall magnetic resonance imaging (vwMRI) were performed within 14 days after symptom onset. The exclusion criteria were: 1) other vasculopathies, such as dissection and vasculitis.; 2) incomplete image data or poor image quality.

3.2.2 CT protocol and post-processing

A routine non-enhanced brain CT was performed using a 64-slice multi-detector row CT system (Light speed 64 plus, General Electric, Milwaukee, WI, USA) with acquisition parameters as follows: slice thickness 5 mm; 120 kVp, 170 mAs, 1s per rotation. All axial images were reconstructed at 0.625-mm intervals. Each CT scan was obtained in axial mode, with tilting along the occipital-meatal line.

The presence and patterns of IAC were assessed on reconstructed CT images by two readers (H.D. and L.Z.) who had more than 3 years of experience in brain CT imaging and were blinded to the clinical and MRI data. Each major intracranial artery segment was assessed, including the cavernous segment (C4) of the internal carotid artery (ICA), the supraclinoid and ophthalmic segment (C5-6) of the ICA, M1 segment of the middle cerebral artery (MCA), intracranial segment (V4) of the vertebral artery (VA), and the basilar artery (BA). IAC was defined as hyperdense foci over 130 Hounsfield units (HU) (refs). The patterns of IAC were categorized into intimal or medial according to a previously established scoring model[21], in which circularity (1 for dot, 2 for <90°, 3 for 90-270° and 4 for 270-360°), thickness (1 for thick IAC ≥ 1.5 mm and 3 for thin IAC < 1.5 mm) and morphology along the long axis of the artery (0 for indistinguishable, 1 for irregular/patchy and 4 for continuous) were evaluated and summed up as a total score (**Figure 3-1**). A total score that ranged from 1 to 6 indicated predominantly intimal calcification (intimal IAC) and 7 to 11

was deemed as predominantly medial calcification (medial IAC).

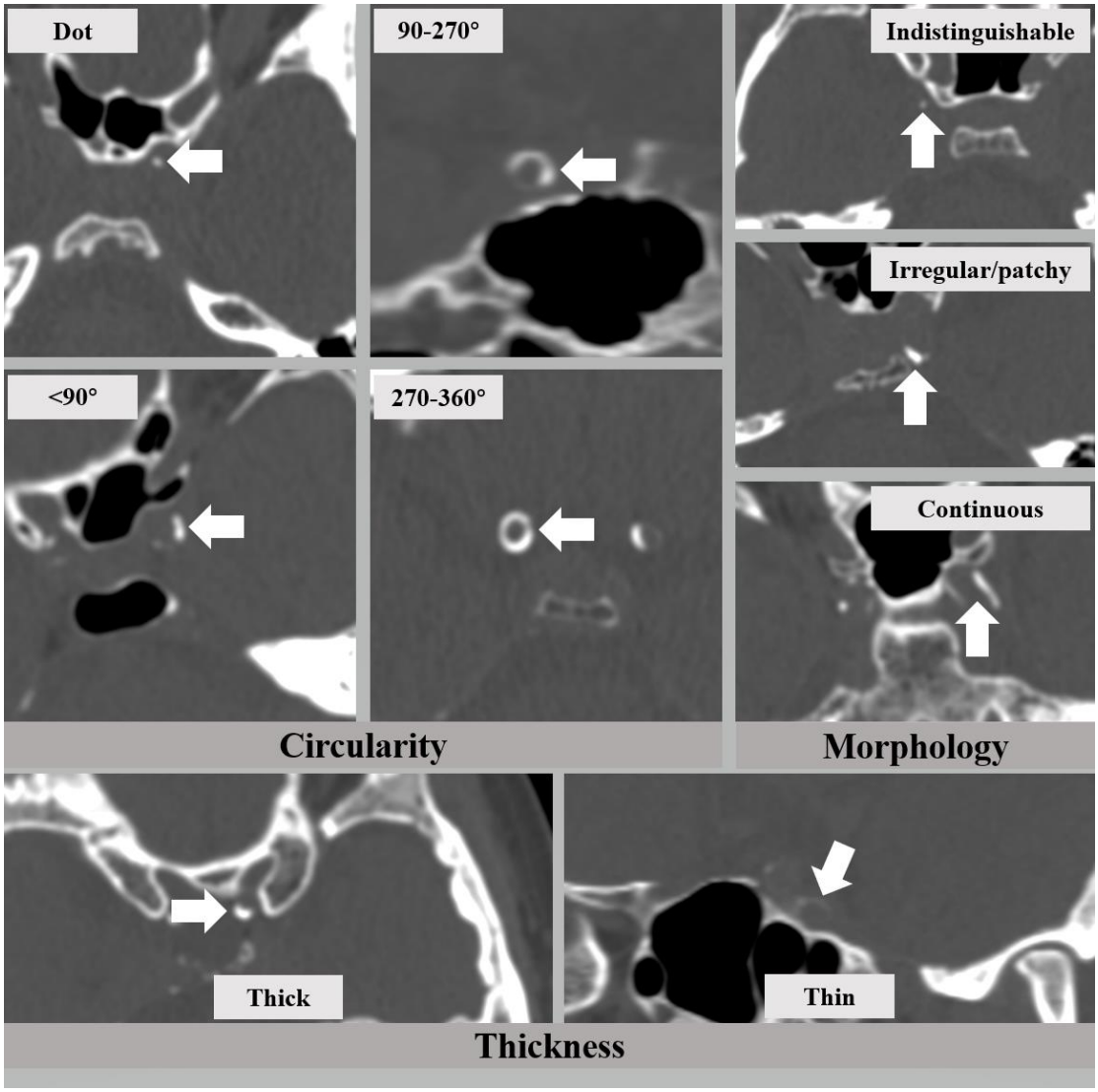

Figure 3-1. The intracranial arterial calcification (IAC) grading scale to differentiate IAC pattern is based on the circularity, thickness and morphology of IAC.

3.2.3 MRI protocol and post-processing

Vessel-wall MRI was performed using a 3.0 Tesla Achieva MR system (Philips Healthcare, Cleveland, OH, USA) with an 8-channel head coil. A time-of-flight MR angiography (TOF-MRA) sequence and a transverse 3D T1-weighted volumetric isotropic turbo spin-echo acquisition (T1w-VISTA) sequence were acquired. Parameters for TOF-MRA were as follows: field-of-view (FOV) 200 × 200 × 56 mm^3, acquired resolution 0.4 × 0.6 × 0.7 mm^3, repetition time (TR)/echo time (TE) 23/3.5 ms. Parameters for T1w-VISTA: FOV 200 × 167 × 45 mm^3, acquired resolution 0.6 × 0.6 × 1.0 mm^3, reconstructed resolution 0.5 × 0.5 × 0.5 mm^3, TR 1,500 ms, TE 36 ms. vwMRI images were repeated after the injection of gadolinium-containing contrast agent (Dotarem; Guerbet, Roissy CdG Cedex, France) intravenously with a ratio of 0.2 ml/kg and an injection rate of 3.5 ml/s.

The presence of atherosclerotic lesion was identified on reconstructed images (**Figure 3-2**) using the OsiriX DICOM Viewer (Pixmeo SARL, Bernex, Switzerland) by two readers (H.D. and L.Z.) who were blinded to the clinical data and CT findings. Images from TOF-MRA were used for identifying luminal stenosis. Stenosis was evaluated on reconstructed images and maximum intensity projection (MIP) images with four degrees: (1) mild stenosis, (2) moderate stenosis, (3) severe stenosis and (4) signal void. Images from vwMRI were reconstructed perpendicular to the axis of the vessel by OsiriX DICOM Viewer (Pixmeo SARL, Bernex, Switzerland). An atherosclerotic plaque was defined as vessel wall thickening on pre- and post-contrast vwMRI. The outer-wall area (OWA) and lumen area (LA) were measured manually by tracing the outer interface and lumen-intima interface, respectively (**Figure 3-3**). Plaque burden, maximum and minimum wall thickness were derived accordingly (**Figure 3-4**). Plaque burden was defined as (OWA-LA)/OWA. Eccentricity index was defined as (maximum wall thickness−minimum wall thickness)/maximum wall thickness. A plaque was defined as eccentric if the index was ≥0.5 or concentric if <0.5, as

described in a prior study[142]. Intraplaque hemorrhage (IPH) was defined an area of hyperintensity (>150% of the adjacent area of the vessel wall) within the plaque (**Figure 3-3**)[143].

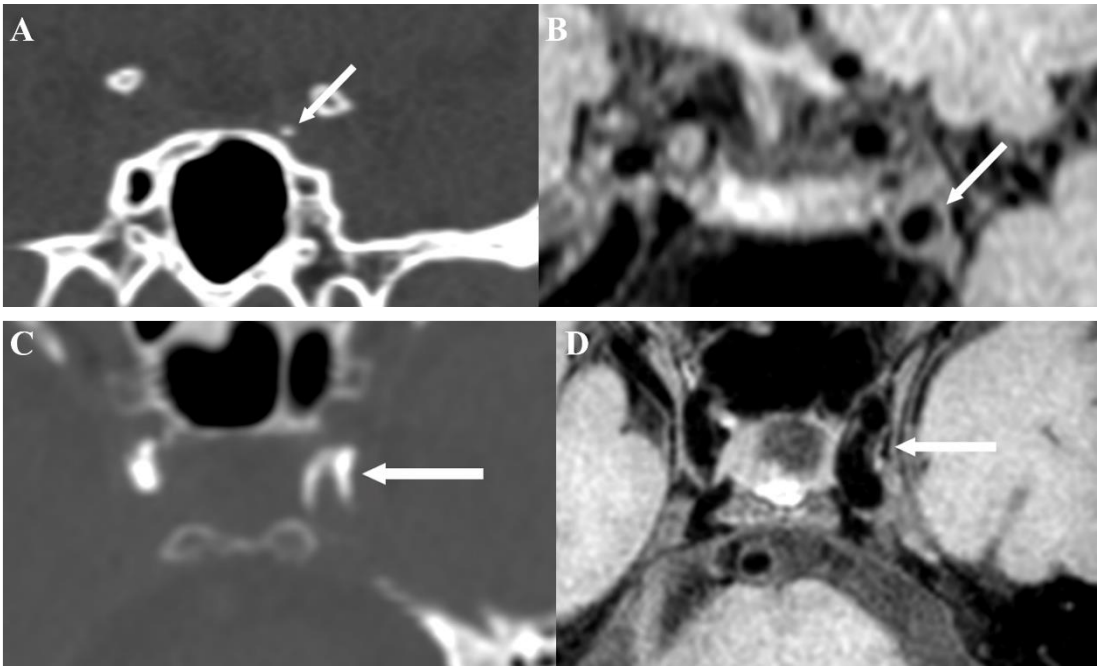

Figure 3-2. Intimal calcification detected by brain CT (A) and the corresponding site on vessel-wall magnetic resonance (vwMRI) (B). Medial calcification detected by brain CT (C) and the corresponding site on vwMRI (D).

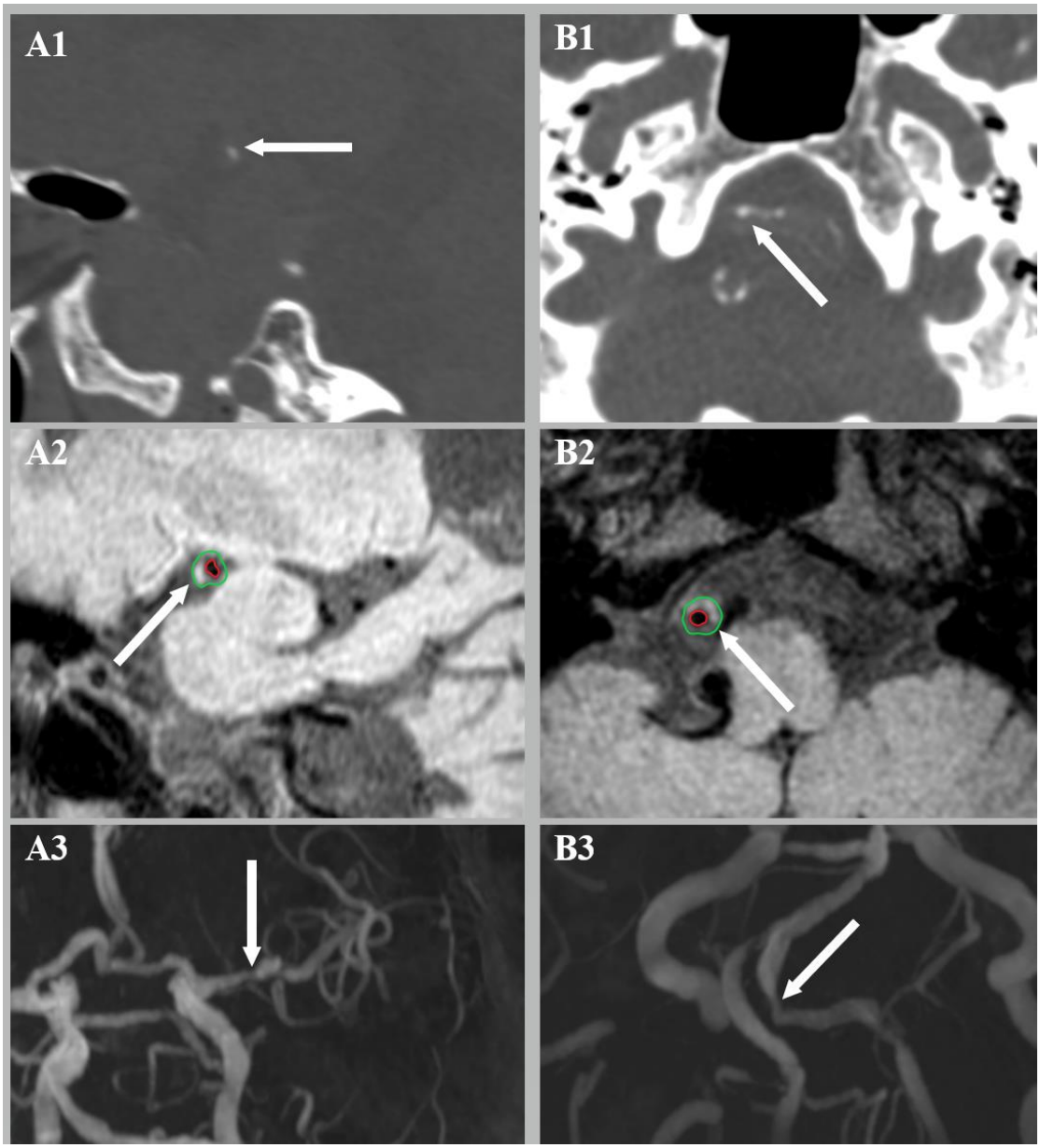

Figure 3-3. Intraplaque hemorrhage (A2) and luminal stenosis (A3) were detected at the corresponding site of intimal calcification (A1). Medial calcification (B1) was found with concurrent intraplaque hemorrhage (B2) and luminal stenosis (B3).

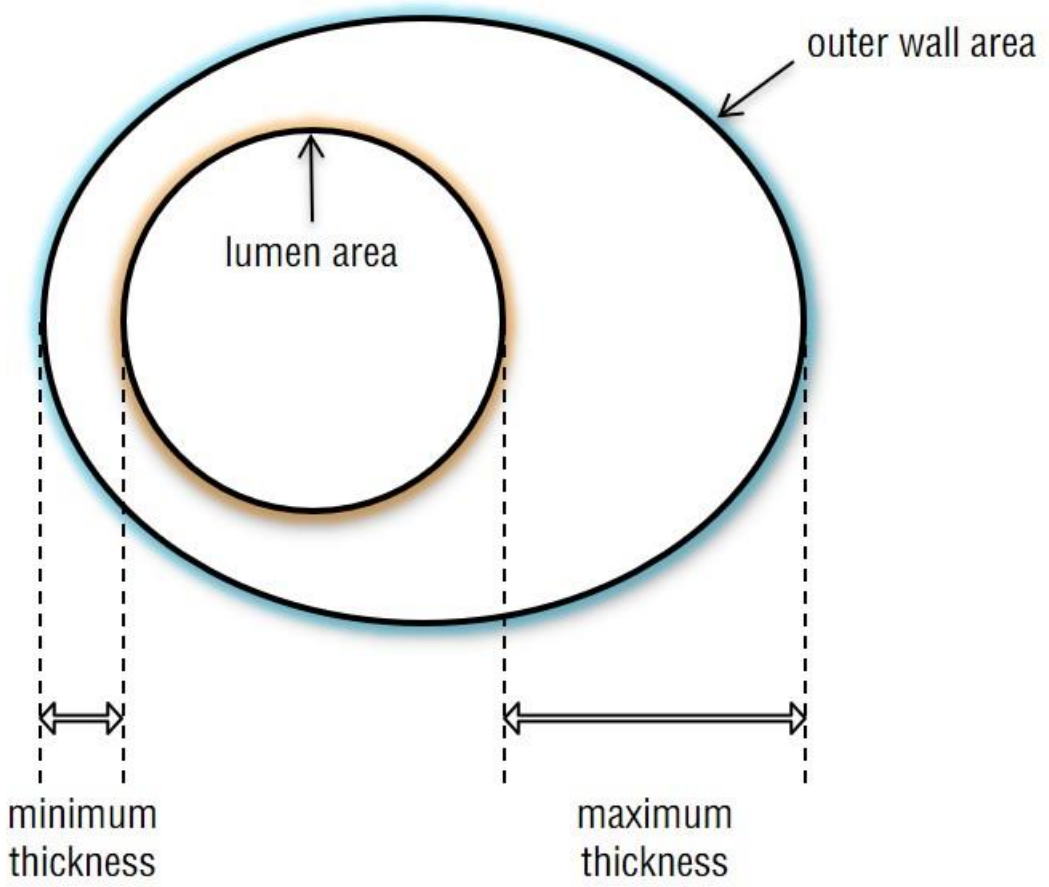

Figure 3-4. The measurement of intracranial arterial plaque. Plaque burden = (outer wall area – lumen area) / outer wall area. Eccentricity = (maximum thickness – minimum thickness) / maximum thickness.

3.2.4 Imaging matching

After the identification of IAC on CT and plaques on vwMRI, a side-by-side analysis was conducted to check whether the stenosis and plaques were at the corresponding site of IAC based on anatomy, as showed in **Figure 3-2**.

3.2.5 Statistical analysis

For statistical analysis, IBM SPSS (20.0, SPSS, Inc) was used. Continuous variables were expressed by mean ± SD and categorical variables were presented as numbers and percentages. *T*-test was used for comparison of age and plaque burden between groups. Pearson's Chi-square test or Fisher's exact test was used for comparisons of the distribution patterns of IAC, category of luminal stenosis, stenotic degree, eccentricity and IPH between plaques with intimal calcification and that with medial calcification. Multiple regression was used to evaluate the association between IAC and luminal stenosis, eccentricity, and IPH. A two-sided $p < 0.05$ was considered statistically significant.

3.3 Results

3.3.1 Baseline characteristics

A total of 79 patients were included and four patients were excluded due to incomplete images or poor image quality. It was found that 69 (87.3%) patients had IAC in one or more major intracranial arteries, and the other 10 patients had no calcifications. Patients with IAC were generally older than those without IAC (mean age, 65.3 ± 10.7 vs. 54.1 ± 8.7 years, p=0.001).

Among patients with IAC, 44 out of 69 (63.8%) patients were men (mean age, 64.0 ±

11.2 years). History of smoking was found in 22 out of 69 patients (31.9%). In addition, 54 out of 69 patients (78.3%) had hypertension while the prevalence of diabetes and hyperlipidemia was 39.1% and 50.7%, respectively. Among 10 patients without IAC, the number of men was four. Of the 10 patients, six had history of smoking, seven had hypertension, two had diabetes and three had hyperlipidemia.

3.3.2 Distribution of intimal IAC and medial IAC

Among the 69 patients with IAC, 65 (94.2%) had 1 to 4 calcifications and one patient had as many as 7 calcified arteries. A total of 460 segments were examined, out of which 161 segments (35.0%) with either intimal or medial calcification were identified on brain CT (**Table 3-1**). Most IACs were located in the cavernous (37.9%) and supraclinoid-to-ophthalmic (34.2%) segment of ICA, followed by the V4 segment of the VA (20.5%), M1 segment of the MCA (5.0%), and the BA (2.5%).

Of the 161 IACs, 99 (61.5%) were categorized as predominantly intimal IACs and the other 62 (38.5%) as predominantly medial IACs (**Table 3-1**). **Figure 3-5** shows the distribution of the cumulate calcification score. Among intimal IACs, 48 were given a score of 5, accounting for the majority (48.5%). A score of 4 was identified in 20 intimal IACs (20.2%), followed by a score of 6 in 19 intimal IACs (19.2%) and a score of 3 in 12 intimal IACs (12.1%). Among medial IACs, a score of 10 was more prevalently identified than others (23 in 62 cases, 37.1%). The occurrences of the scores of 7 (12 in 62, 19.4%), 9 (10 in 62, 16.1%) and 11 (14 in 62, 22.6%) were close. Only 3 cases were given a score of 8.

Intimal IAC was frequently detected in the cavernous (34.3%) and supraclinoid-to-ophthalmic (30.3%) segments of ICA and the V4 segment of the VA (24.2%), while medial IAC was more prevalently present in the cavernous (43.5%) and supraclinoid-to-ophthalmic

(40.3%) segment of the ICA. Compared with intimal IACs, medial IACs were more predominantly distributed in the C4-C6 segment of the ICA (64.6% vs. 83.9%, p=0.008) and less frequently involved the intracranial VA (p=0.137).

Table 3-1. Distributions of intracranial arterial calcification (IAC) and IAC with intracranial atherosclerotic disease (ICAD) in major intracranial artery segments.

	Calcification			Calcification with ICAD
	Intimal	Medial	Total	
ICA				
C4	34 (34.3%)	27 (43.5%)	61 (37.9%)	38 (31.9%)
C5-6	30 (30.3%)	25 (40.3%)	55 (34.2%)	39 (32.8%)
M1	7 (7.1%)	1 (1.6%)	8 (5.0%)	8 (6.7%)
V4	24 (24.2%)	9 (14.5%)	33 (20.5%)	30 (25.2%)
BA	4 (4.0%)	0 (0.0%)	4 (2.5%)	4 (3.4%)
Total	99	62	161	119

ICA, internal carotid artery; C4, cavernous segment; C5-6, supraclinoid and ophthalmic segment; M1, M1 segment of middle cerebral artery; V4, V4 segment of vertebral artery; BA, basilar artery

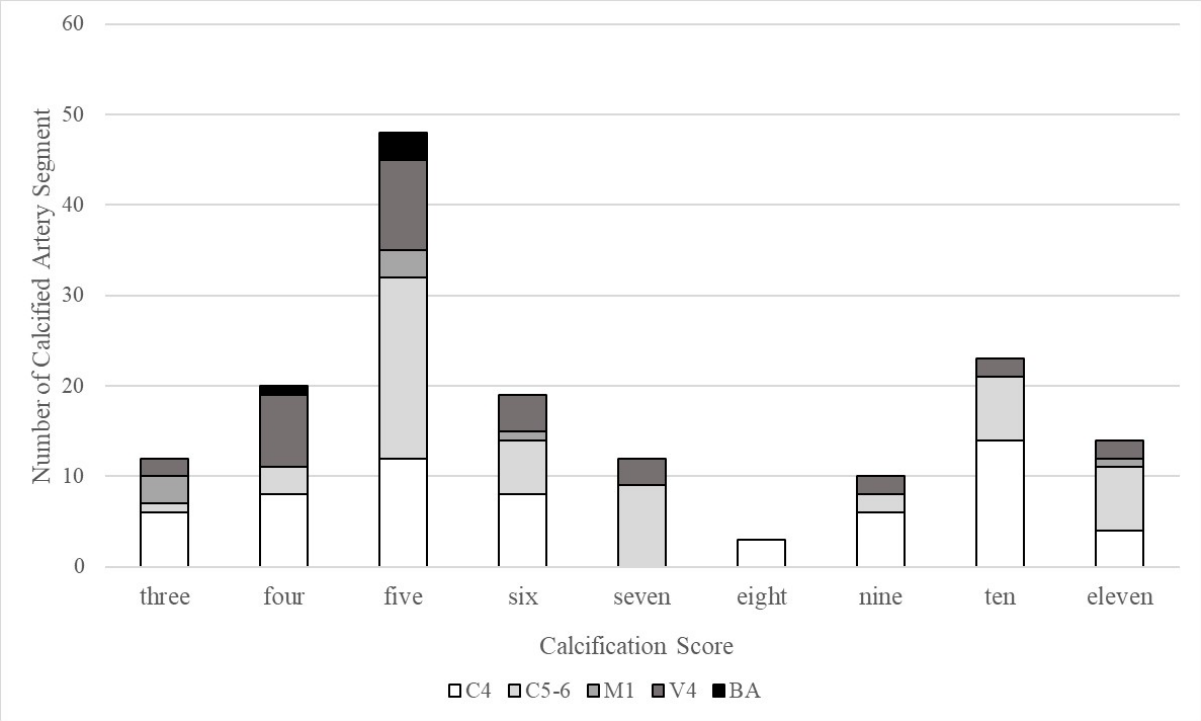

Figure 3-5. Distribution of the cumulate calcification scores. C4, cavernous segment of internal carotid artery; C5-6, supraclinoid and ophthalmic segment of internal carotid artery; M1, M1 segment of middle cerebral artery; V4, V4 segment of vertebral artery; BA, basilar artery

3.3.3 Imaging characteristics of ICAD with intimal or medial calcification

Corresponding intracranial artery segments from 10 patients without IAC were included as control. A total of 70 calcium-free segments were examined, of which 40 (57.1%) were identified as "non-calcified plaques".

Comparing atherosclerotic lesions assessed by vwMRI, 79.8% of intimal IACs (79/99) co-existed with atherosclerotic plaque while 64.5% of medial IACs (40/62) had concurrent plaque. Luminal stenosis was more frequently found in plaques with intimal IAC than plaques with medial IAC (74.7% vs. 47.5%; p=0.003) and non-calcified plaques (74.7% vs. 45.0%; p=0.001) (**Table 3-2**). There was no significant difference in the category of stenosis ("mild and moderate stenosis" vs. "severe stenosis and signal void") among plaques with intimal IAC, plaques with medial IAC, and non-calcified plaques. More plaques with intimal IAC showed as eccentric than plaques with medial IAC (67.1% vs. 45.0%; p=0.02) and non-calcified plaques (67.1% vs. 42.5%; p=0.01). Plaques with intimal IAC had higher plaque burden compared with plaques with medial IAC (0.718 ± 0.104 vs. 0.644 ± 0.122, p=0.001) and non-calcified plaques (0.718 ± 0.104 vs. 0.656 ± 0.104, p=0.003). IPH was found in 35.4% of plaques with intimal IAC, much higher than that in plaques with medial IAC (7.5%, p=0.001) and non-calcified plaques (15.0%, p=0.02). No difference in the presence of luminal stenosis, plaque burden, eccentric pattern or IPH was found between plaques with medial IAC and non-calcified plaques.

The multiple binary logistic regression model revealed that the presence of luminal stenosis (*OR* 2.140; 95% *CI*, 1.001-4.578; p=0.05), eccentric plaque (*OR* 2.621; 95% *CI*, 1.376-4.993; p=0.003), and IPH (*OR* 3.269; 95% *CI*, 1.376-7.767; p=0.007) were associated with intimal IAC, after adjusting for age, gender, history of hypertension, diabetes, hyperlipidemia, and smoking.

Table 3-2. Vessel wall morphology of arterial segments with intracranial atherosclerotic disease (ICAD) and concurrent intimal (or medial) pattern of IAC and arterial segments with non-calcified atherosclerotic plaques.

Change in vessel wall	ICAD coexisting with IAC		
	ICAD plus intimal IAC	**ICAD plus medial IAC**	***Non-calcified plaque**
Total	79	40	40
Vessel stenosis			
Total stenotic segments	59 (74.7%)	19 (47.5%)	18 (45.0%)
Mild-to-moderate stenosis	35 (44.3%)	14 (35.0%)	13 (32.5%)
Mild	22 (27.8%)	8 (20.0%)	9 (22.5%)
Moderate	13 (16.5%)	6 (15.0%)	4 (10.0%)
Severe stenosis and signal void	24 (30.4%)	5 (12.5%)	5 (12.5%)
Severe	12 (15.2%)	3 (7.5%)	3 (7.5%)
Signal void	12 (15.2%)	2 (5.0%)	2 (5.0%)
Plaque burden	0.718 ± 0.104	0.644 ± 0.122	0.656 ± 0.104
Plaque morphology			
Concentric plaque	26 (32.9%)	22 (55.0%)	23 (57.5%)
Eccentric plaque	53 (67.1%)	18 (45.0%)	17 (42.5%)
Intraplaque hemorrhage	28 (35.4%)	3 (7.5%)	6 (15.0%)

**Non-calcified plaque was identified via vessel-wall magnetic resonance imaging among 70 major intracranial artery segments from 10 patients without intracranial arterial calcifications on brain CT.*

3.4 Discussion

In the present study, we found that medial calcifications were prevalently distributed in the intracranial ICA and VA while intimal calcifications could be detected in all major intracranial arteries. Compared with plaques coexisting with medial IAC and non-calcified plaques, plaques with intimal IAC was more associated with the presence of luminal stenosis, eccentric plaques, higher plaque burden, and the presence of IPH.

A prior CT study reported that the intracranial ICA was the most frequently affected segment by IAC (60%), whereas the MCA and the BA were rarely affected (5%, respectively)[72]. A possible reason of the high prevalence of IAC around the cavernous and carotid siphon may be the tortuous anatomical configuration, which may result in abnormal hemodynamics[77]. In the present study, medial calcifications were found predominantly distributed in the intracranial segment of the ICA and the VA, consistent with previous histopathological findings [8,9]. It is noteworthy that a prior autopsy study reported an extremely high prevalence of calcification (97.4%) along the medial layer in the intracranial ICAs[9] and a relatively high prevalence of medial calcification (36.8%) among the intracranial VAs[8]. In the present study, such high prevalence was not found in either the ICA or the VA. As reported in the autopsy, 36% of the medial calcifications had calcified circumference <50%[9]. With less circumference affected, medial IAC may have cluster-like shape instead of circular shape, which may be categorized as intimal calcification on CT.

Histopathological findings demonstrated that intimal IAC was present prevalently in the advanced stage of atherosclerosis[8]. Being granules initially, intimal calcification will grow in size with constant fusion and will turn into large clusters[56]. Previous autopsy study revealed a marked concurrence of atherosclerotic lesion among intimal calcifications (85%) but not vice-versa (62%) in the carotid artery[9], partly matching the concurrence of medial

calcification and intracranial atherosclerosis (64.5%) in the present study. Although medial IAC is thought to form independently of the presence of atherosclerosis, concurrent intracranial plaques was in 38% of medial IACs on histology [8]. Luminal stenosis, prevalently presented in ICAD and resulting in multiple stroke patterns[6], is one of the most critical causes of severe stroke and recurrent strokes[105,144]. In the present study, intimal IAC was found independently associated with the presence of luminal stenosis. However, no significant difference in stenotic severity was identified between atherosclerotic lesions with intimal or medial IACs. Previous histological findings suggested that among major cerebral arteries the presence of intimal IAC was associated with more severe luminal stenosis[8]. Since the medial IACs in the comparison of our study were concurrent with atherosclerosis while the referred histological study included all medial IACs (with or without atherosclerosis)[8], the prevalence of narrowed artery might be increased in our study.

With luminal stenosis exceeding over 70%, eccentric plaque in the extracranial carotid artery becomes associated with a significant increased incidence of ipsilateral stroke[145]. Similar findings were found in intracranial arteries. Most of the progressive ICAD presented eccentric plaques (84.4%)[146], which was much more prevalently than other kinds of vasculopathy[147]. However, discrepancies exist. First, the prevalence of eccentric plaque differs between anterior circulation and posterior circulation[148]. Second, eccentricity was reported either associated[149] or not[150] with stroke according to different studies. Furthermore, a histological study demonstrated a similar proportion of eccentric and concentric lesions among ICAD of general adults[151]. One of the causes might be the inclusion criteria: progressive ICAD are always accompanied by luminal stenosis[147] while recruited samples from general adults[151] may consist of lesions from different stages of ICAD. The process of change in histological feature during the evolvement of atherosclerosis can explain some of the discrepancies and more studies upon eccentricity are required to clarify its relevance to

ICAD and strokes. Except for eccentricity, plaque burden is another notable factor that tends to be underestimated by traditional lumen angiographic imaging as a result of vessel wall enlargement induced by positive vascular remodeling during the progress of ICAD[149]. Previous study reported the association of plaque burden with the National Institutes of Health Stroke Scale (NIHSS) scores[152], indicating a latent impact of plaque burden on stroke severity. Additionally, a recent vwMRI study revealed that plaque burden was significantly correlated to recurrent stroke with every 10% increase in plaque burden leading to a 2.26-fold higher risk of stroke recurrence[153]. By connecting IAC with eccentricity and plaque burden, the present study was thought to provide an improvement in assessing the risk and prognosis of ischemic stroke from the other side.

Intraplaque hemorrhage, attributed to immature and fragile neovessels[154], is an important marker of plaque vulnerability and predictor of ischemic events[75,99,155]. By a sudden expansion in plaque components, IPH can exacerbate luminal stenosis, or worse, result in plaque rupture, leading to in situ thrombosis and distal embolism[156]. In prior studies, correlation of IAC and plaque vulnerability was controversial[13,14]. A latent reason for such controversy could be the unclassified IAC patterns. The present study provided additional evidence by identifying intimal calcification as an independent risk factor for IPH. In calcified coronary arteries with plaque rupture, the junctions between superficial calcified nodules and soft plaque were vulnerable to shear stress that may cause plaque rupture[97]. Similar findings were reported in terms of carotid arteries[104]. Since intimal calcifications are present in the superficial layer of the vessel wall, we deduce that the possible mechanism of IAC-induced IPH could be the deformation and rupture of intraplaque neovessels resulting from elevated shear stress upon the plaque surface. To test this hypothesis, investigations on the mechanical conditions and hemodynamics of IAC might be needed.

The organization of intracranial arteries have unique features compared with other

vessels: a thinner muscular layer, a less abundant adventitia and a hypoplastic external elastic membrane[43]. These features make intracranial arteries frailer under external pressure than other vessels. During mechanical endovascular thrombectomy or arterial stenting, one concern is arterial calcification. The presence of calcification is associated with arterial stiffness[110], which is thought to make vessel wall more brittle during endovascular procedures. As a result, proper surgical instruments, delicate control of balloon dilatation during stenting (or thrombectomy) will significantly lower the risk of unexpected vessel rupture. Another concern could be the smoothness of stent retrieving during endovascular thrombectomy. Large calcified lesions or stiffened vessel wall may block the extraction of thrombus and therefore increase the passes of retriever, leading to prolonged procedure time and worse outcome[18]. According to our study, intimal IAC more often coexists with luminal stenosis, larger plaque burden, eccentric plaque and IPH. By distinguishing the pattern of IAC, we aimed to provide evidence for decision making in pre- and post-treatment assessments. Future studies will also be needed to investigate the latent impacts of IAC pattern on the pathophysiological process of stroke.

This study had limitations. First, it was a relatively small cohort. The sample size limited the degrees of freedom and adjustable variables, thereby leading to potential overestimation. Second, all patients had either infarction or TIA, of whom the prevalence of IAC and progressive ICAD could be higher than that among general adults. Thirdly, during the assessment of IAC, it was found that some of medial IAC were allocated scores close to the definition of intimal. It suggested that some of the early-stage medial IAC might be categorized as intimal IAC. Furthermore, as being part of another study with specific inclusion and exclusion criteria and long period, the cohort is based hence not representative of the true prevalence of IAC. In addition, as not all the patients in this cohort had post-contrast T1 sequence, the correlation between IAC pattern and contrast enhancement was not

studied, which may limit the interpretation of our findings. By far, it is still difficult to link IAC to territorial stroke by the findings in our study. Except for ICAD in the carotid artery, MCA lesions also takes a dominant role in the ischemic events of anterior circulation. Under the coexistence of multiple atherosclerotic lesions in both ICA and MCA, it is difficult to identify the culprit lesion that accounts for infarction (or TIA), especially artery-to-artery embolism and hypoperfusion. On the other hand, as single subcortical infarction is prevalently led by occluded perforating artery of MCA, its relevance to ICA calcification seems loose. Whether IAC has impact on territorial infarction requires further studies.

3.5 Conclusions

The present multimodal imaging-based study revealed both similitude and difference between ICAD and IAC. Medial IAC is prevalently distributed along carotid siphon. Compared with plaques with medial IAC and non-calcified plaques, plaques with intimal IAC more frequently shows luminal stenosis, eccentric plaque, larger plaque burden and IPH, indicating a latent impact of intimal IAC on plaque vulnerability. Our findings may provide evidence for clinical evaluation on mechanism, risk and prognosis of ischemic stroke. The understanding of the mechanism in the presence of calcification will be deepened with further study upon the correlation of IAC and territorial infarctions.

Chapter 4. Substudy-2: Correlation between Medial Pattern of Intracranial Arterial Calcification and White Matter Hyperintensities

Chapter 4. Substudy-2

4.1 Background

Intracranial arterial calcification (IAC) is found to be an independent risk factor for stroke[11,121]. Apart from its association with intracranial large artery atherosclerosis[12,51], IAC may also correlate to cerebral small vessel disease (CSVD)[157,158], of which white matter hyperintensities (WMH) is one of the common types on neuroimaging[159]. However, current studies about the impact of IAC on WMH are controversial. Despite several reports showing that the presence[73] and the density[83] of IAC may be associated WMH, opposite findings also suggested irrelevance between IAC and WMH[160]. IAC mainly involves the intima and the media of intracranial arterial walls[8,9]. Histological and clinical evidence suggested that intimal IAC is associated with atherosclerosis[8] while medial IAC may have impact on arterial stiffness[161].

By far, little has been studied on the association between IAC pattern and WMH. We hypothesized that IAC patterns may be correlated with WMH differently. In the present study, we aimed to investigate the association between IAC pattern (intimal IAC and medial IAC) and the presence and burden of WMH.

4.2 Methods and materials

4.2.1 Subjects

This study was approved by the Clinical Research Ethics Committee of the Peking University Shenzhen Hospital. Consecutive patients admitted to the stroke center from November 2020 to April 2022 were recruited retrospectively. The inclusion criteria were: 1) patients above 18 years old who had acute ischemic stroke or transient ischemic attack (TIA); 2) non-contrast brain computed tomography (CT) and magnetic resonance imaging (MRI) were performed within 7 days after symptom onset. The exclusion criteria were as follows: 1)

contraindications to either CT or MRI; 2) other causes of white matter lesions, including multiple sclerosis, vasculitis, or connective tissue diseases; 3) patients with critical medical condition, such as head trauma and brain tumor; 4) poor quality of imaging data and incomplete clinical data.

Baseline characteristics including age, smoking, drinking, diabetes, hypertension, hyperlipidemia, atrial fibrillation, kidney function as well as the level of renal impairment, previous stroke or transient ischemic attack (TIA) and history of ischemic heart disease were collected for each patient. Smoking was defined as current user of cigarettes. Drinking was defined as having a daily intake of alcohol. Diabetes was defined as fasting blood glucose level ≥ 7 mmol/L in two independent tests. Hypertensions was defined as either systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg in resting state. Hyperlipidemia was defined as serum total cholesterol ≥ 5.2 mmol/L or serum low density lipoprotein cholesterol ≥ 3.4 mmol/L or serum triglyceride ≥ 1.7 mmol/L. Atrial fibrillation was defined as the absence of regular P-wave with irregular R-R interval, examined by electrocardiogram. Kidney function was assessed by estimating estimated glomerular filtration rate (eGFR). Serum creatinine (sCr) was examined in each subject for calculating eGFR using the modified glomerular filtration rate estimating equation for Chinese populations: eGFR (mL/min/1.73m^2) = 175 × (sCr)-1.234 × (age)-0.179 (× 0.79 if female). Normal kidney function was defined as eGFR > 90 mL/min/1.73m2; mildly reduced kidney function was defined as eGFR 60-90 mL/min/1.73m2 and decreased kidney function was defined as eGFR < 60 mL/min/1.73m^2. Previous stroke/TIA was defined as at least single episode of either ischemic stroke or TIA before the inclusion of the present study. Ischemic heart disease was defined as history of coronary atherosclerotic heart disease, with or without having percutaneous coronary stenting.

4.2.2 Assessment of intracranial arterial calcification

Intracranial arterial calcification (IAC) was assessed by brain CT. Major intracranial artery segments including the lacerum and cavernous segment (C3-C4) of internal carotid arteries (ICAs), the supraclinoid segment to the communicating segment (C5-C7) of ICAs, M1 segment of middle cerebral arteries (MCAs), proximal and distal part of the intracranial (V4) segment of vertebral arteries (VAs) and the basilar artery (BA) were examined. CT images were assessed by two neurologists with more than 5 years' experience of neuroimaging (XL.L. and H.D.) who were both blinded to clinical characteristics and MRI images. The presence of IAC was defined as hyperdense foci over 130 Hounsfield units (HU). Based on a previously developed and validated grading scale[21], IAC was first categorized into two patterns: intimal IAC and medial IAC (**Figure 4-1**). Circularity (1 for dot, 2 for <90 degrees, 3 for 90-270 degrees and 4 for 270-360 degrees), thickness (1 for thick IAC ≥ 1.5mm and 3 for thin IAC < 1.5mm) and morphology (0 for indistinguishable, 1 for irregular/patchy and 4 for continuous) were assessed and graded. A summed score from 1 to 6 was defined as predominant intimal IAC and 7 to 11 was defined as medial IAC. Based on the categorization, IAC was further evaluated by its count and involvement. The number of arteries involved by IAC (either intimal or medial) was defined as the summed number of the examined artery segments that had IAC (either intimal or medial). The quartiles (Q1, Q2 and Q3) of artery numbers were calculated and used subsequently for grouping. The severity of IAC was defined as focal or diffuse (**Figure 4-2**): focal IAC was defined as the trajectory of IAC involving less than 1/3 of the examined artery segment; diffuse IAC was defined as the trajectory of IAC involving more than 1/3 of the examined artery segment.

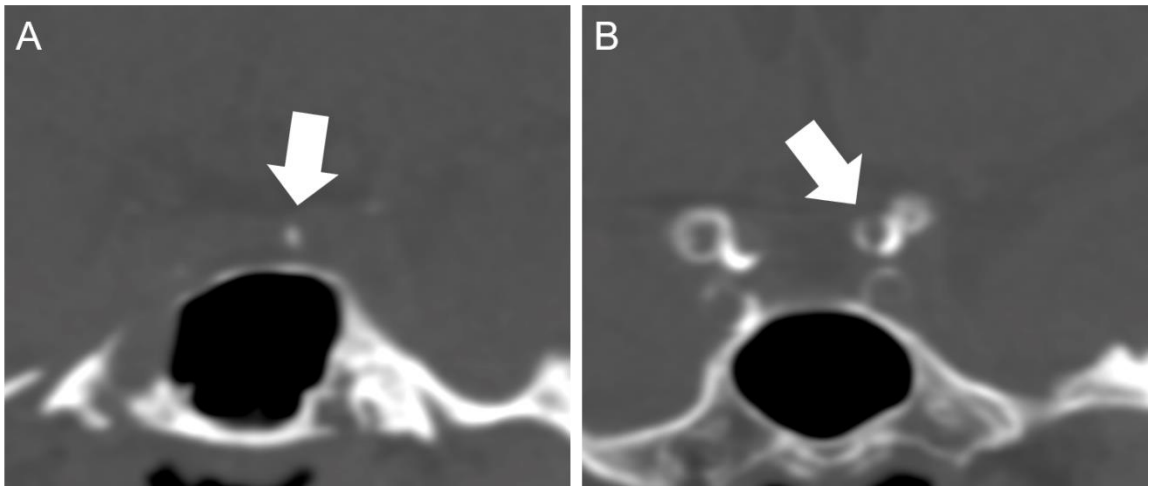

Figure 4-1. Intracranial arterial calcification (IAC) pattern on computed tomography (CT). Intimal IAC (A) as a patchy cluster and medial IAC (B) extending up to the whole circumference of vessel wall.

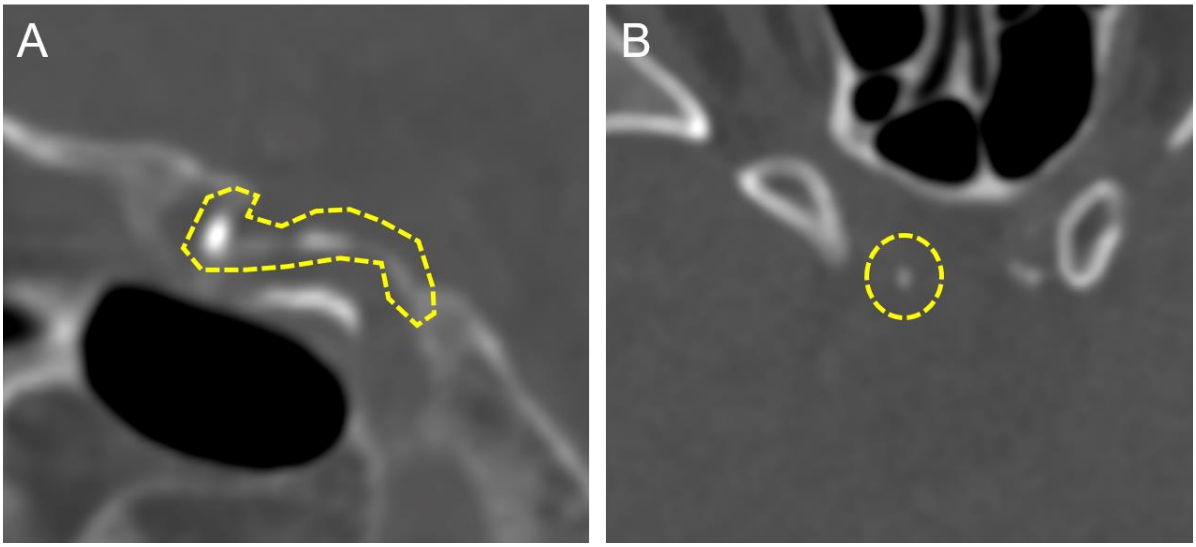

Figure 4-2. Different severity of intracranial arterial calcification (IAC). Diffuse IAC (A) extended over 1/3 of the length of the examined intracranial artery segment while focal IAC (B) involved less than 1/3 of the examined intracranial artery segment.

4.2.3 Evaluation and grading of white matter hyperintensities

The evaluation of WMH was based on brain T2w and T2 fluid attenuated inversion recovery (T2-FLAIR) images. Diffusion weighted imaging (DWI) and apparent diffusion coefficient (ADC) were used to exclude acute infarct lesions. MRI Images were assessed by two independent raters with more than 5 years of experience in brain MRI (H.D. and YJ.D.) who were blinded to clinical characteristics and CT images. White matter hyperintensities (WMH) was graded according to a previously published and validated 8-grade scale[162,163]. Grade 1 referred to discontinuous periventricular rim with minimal dots of subcortical lesions; Grade 2 was thin and continuous periventricular rim with a few patchy subcortical lesions; Grade 3, thicker, continuous periventricular rim with scattered subcortical lesions; Grade 4, thicker, shaggier periventricular rim with mild subcortical lesion (possibly with minimal confluent periventricular lesions); Grade 5 represents for mild periventricular confluent lesion that surrounds the frontal horn and occipital horn; Grade 6 was moderate periventricular confluent lesion surrounding the frontal horn and occipital horn; Grade 7 was periventricular confluent lesion with the centrum semiovale involved moderately; Grade 8, periventricular confluent lesion involving most region of the centrum semiovale. Images with no white matter findings were graded 0 and those with WMH more severe than Grade 8 were scored 9. After the grading of WMH, the burden of WMH was categorized as absent, mild, moderate and severe (**Figure 4-3**). “WMH absent” indicated Grade 0. “Mild WMH” consisted of Grade 1 and Grade 2. “Moderate WMH”, included Graded 3 to 5. “Severe WMH” comprised of Graded 6 to 9.

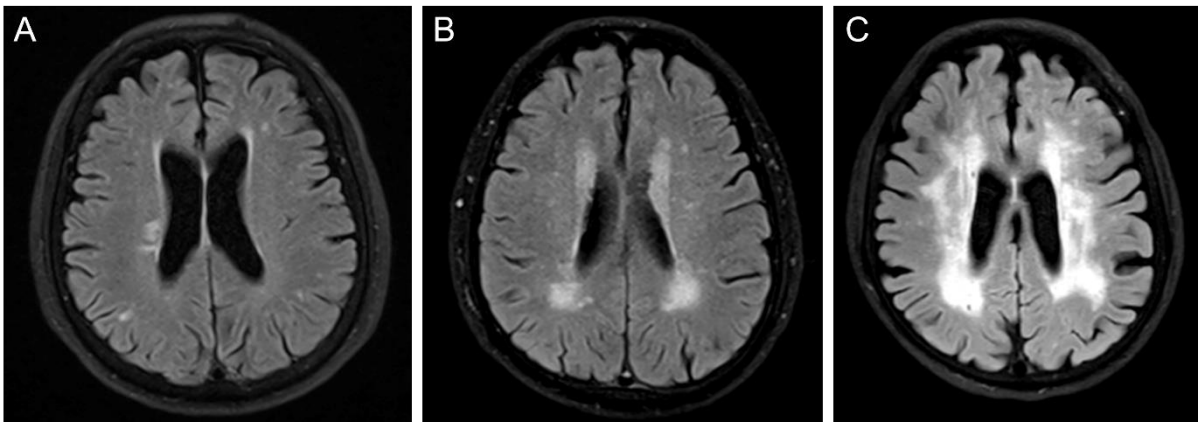

Figure 4-3. The burden of white matter hyperintensities (WMH) was categorized into three levels: mild WMH (A), moderate WMH (B) and severe WMH (C).

4.2.4 Statistical analysis

IBM SPSS (20.0, SPSS, Inc) was used for statistical analysis. Continuous variables were presented as mean ± standard deviation (SD) and categorical variables were presented as numbers and percentages. The inter-rater reliability was assessed by Cohen's kappa analysis. Independent t-test and Mann–Whitney U test were used for continuous variables and Pearson's chi-square test and Fisher's exact test were used for dichotomous categorical variables. Correlations of ordinal variables were measured by Chi-square linear trend test. Vascular risk factors with p value < 0.1 were considered as confounding factors and were tested by collinearity diagnostics. Regression analysis was performed before and after adjusting for confounding factors. Associations between the occurrence of WMH and presence of IAC (intimal or medial) were examined in multiple binary logistic regression models. Multiple ordinal logistic regression analysis was used to evaluate the associations between WMH burden and the presence, involved arteries and severity of medial IAC, respectively. In the ordinal logistic regression models, we set the absence of medial IAC as reference and the results of which were interpreted as comparisons with the absence of medial IAC. A two-sided p less than 0.05 was regarded as statistically significant in the results.

4.3. Results

4.3.1 Baseline characteristics

In total, 265 consecutive stroke patients were included, and 10 patients excluded due to poor image quality or incomplete clinical data. The mean age was 62.26 ± 11.99 years old. 179 patients were men. The clinical baseline information of patients with WMH were presented in **Table 4-1**. Of the 265 patients, 36.2% had history of current smoking and 27.2% had history of drinking. Diabetes was identified in 27.2% of the patients and hypertension was found in

57.0% of the patients. Hyperlipidemia and atrial fibrillation were diagnosed in 16.2% and 4.2% of the patients, respectively. 62 patients had history of previous stroke/TIA and 30 patients had history of ischemic heart disease.

Intracranial arterial calcification was identified in 231 (87.2%) patients. Intimal IAC was present in 169 (54.7%) patients and medial IAC was present in 150 (48.5%) patients. 88 out of 231 patients (38.1%) had coexisting IAC (intimal and medial IAC simultaneously). Diffuse IAC was found in 74 patients, of whom all were medial calcifications. WMH was found in 200 (75.5%) patients, among whom 105 patients had mild WMH, 69 patients were categorized in moderate WMH group and 26 patients were identified to have severe WMH.

Table 4-1. Baseline characteristics of patients with white matter hyperintensities (WMH).

	WMH burden				*p* value
	Absent (n=65)	**Mild (n=105)**	**Moderate (n=69)**	**Severe (n=26)**	
Male, n (%)	48 (73.8)	71 (67.6)	45 (65.2)	15 (57.7)	0.125
Age (mean ± SD, years)	51.34 ± 10.60	62.64 ± 9.812	68.97 ± 9.83	70.23 ± 8.16	<0.001
Smoking, n (%)	31 (47.7)	45 (42.9)	28 (40.6)	8 (30.8)	0.152
Drinking, n (%)	26 (40.0)	33 (31.4)	18 (26.1)	7 (26.9)	0.094
Diabetes, n (%)	17 (26.2)	38 (36.2)	20 (29.0)	9 (34.6)	0.643
Hypertension, n (%)	37 (56.9)	68 (64.8)	52 (75.4)	19 (73.1)	0.026
Hyperlipidemia, n (%)	18 (27.7)	16 (15.2)	12 (17.4)	4 (15.4)	0.146
Atrial fibrillation, n (%)	1 (1.5)	4 (3.8)	7 (10.1)	1 (3.8)	0.107
Previous stroke/TIA, n (%)	6 (9.2)	23 (21.9)	24 (34.8)	9 (34.6)	<0.001
Ischemic heart disease, n (%)	1 (1.5)	13 (12.4)	14 (20.3)	2 (7.7)	0.026
eGFR (mean ± SD, mL/min/1.73 m^2)	106.00 ± 31.39	103.32 ± 31.48	102.45 ± 37.40	105.16 ± 27.56	0.901
Kidney function					

Normal, n (%)	46 (70.8)	65 (61.9)	46 (66.7)	16 (61.5)	
Mildly reduced, n (%)	16 (24.6)	37 (35.2)	16 (23.2)	9 (34.6)	0.351
Decreased, n (%)	3 (4.6)	3 (2.9)	7 (10.1)	1 (3.8)	

SD, standard deviation. TIA, transient ischemic attack.

4.3.2 Association between IAC pattern and WMH

The presence of medial IAC was found to be associated with the presence of WMH (p<0.001). **Table 4-2** showed the more correlations after categorizing the burden of WMH. Patients with medial IAC were more prone to have higher WMH burden (p<0.001). However, compared with medial IAC, intimal IAC was not correlated with the presence of WMH or the burden of WMH (**Figure 4-4**).

The count of medial IAC was found to be associated with the burden of WMH. Patients with higher WMH burden were identified to have higher median number of media IAC count (**Figure 4-5**). After classifying medial IAC count into 3 groups according to the quartiles (**Table 4-2**), the proportion of high WMH burden showed a rising trend among patients with no arteries involved by medial IAC, patients with 1 to 3 arteries involved by medial IAC and patients with at least 4 arteries involved by medial IAC (p<0.001). Similar relationship was found in medial IAC severity (**Figure 4-6**). Patients with more severe medial IAC were more likely to have moderate and severe WMH (**Table 4-2**), compared with patients with milder medial IAC (p<0.001).

Table 4-2. Correlations of two patterns of intracranial arterial calcification (IAC) with white matter hyperintensities (WMH).

	Patients, n	WMH occurrence		*p* value
		Absent, n (%)	Present, n (%)	
Intimal IAC				
Absent	96	28 (29.2)	68 (70.8)	0.186
Present	169	37 (21.9)	132 (78.1)	
Medial IAC				
Absent	115	52 (45.2)	63 (54.8)	<0.001*
Present	150	13 (8.7)	137 (91.3)	

		WMH burden				*p* for trend
		Absent, n (%)	Mild, n (%)	Moderate, n (%)	Severe, n (%)	
Intimal IAC						
Absent	96	28 (29.2)	31 (32.3)	25 (26.0)	12 (12.5)	0.215
Present	169	37 (21.9)	74 (43.8)	44 (26.0)	14 (8.3)	
Medial IAC						

Absent	115	52 (45.2)	51 (44.3)	11 (9.6)	1 (0.9)	<0.001*
Present	150	13 (8.7)	54 (36.0)	58 (38.7)	25 (16.7)	
Medial IAC, involved arteries						
0 arteries	115	52 (45.2)	51 (44.3)	11 (9.6)	1 (0.9)	
1 to 3 arteries	88	12 (13.6)	33 (37.5)	31 (35.2)	12 (13.6)	<0.001*
≥ 4 arteries	62	1 (1.6)	21 (33.9)	27 (43.5)	13 (21.0)	
Medial IAC, severity						
No medial IAC	115	52 (45.2)	51 (44.3)	11 (9.6)	1 (0.9)	
Focal medial IAC	76	10 (13.2)	31 (40.8)	25 (32.9)	10 (13.2)	<0.001*
Diffuse medial IAC	74	3 (4.1)	23 (31.1)	33 (44.6)	15 (20.3)	

*: $p<0.05$

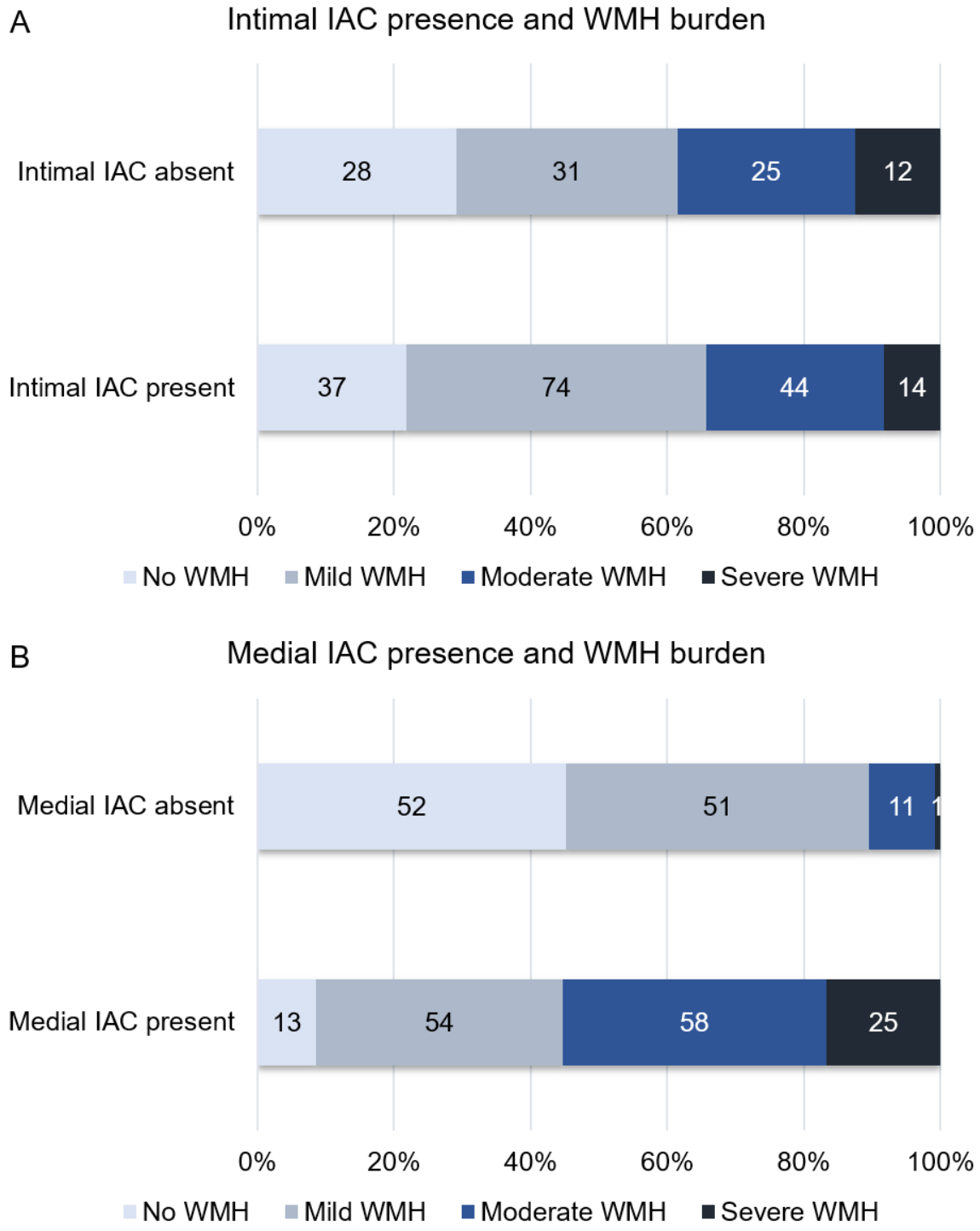

Figure 4-4. Correlations of the presence of two intracranial arterial calcification (IAC) patterns with the burden of white matter hyperintensities (WMH). The presence of intimal IAC is not correlated with the burden of WMH (A). The presence of medial IAC is correlated with higher proportion of moderate and severe WMH (B).

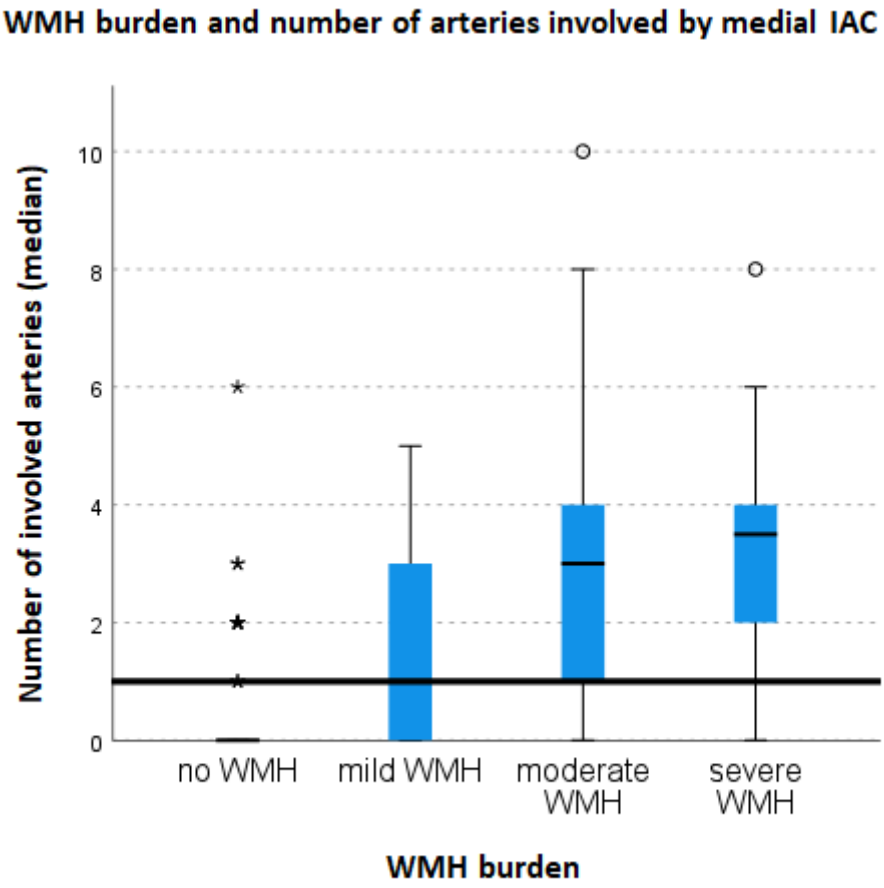

Figure 4-5. The median number of the number of arteries involved by medial IAC in patients with different white matter hyperintensities (WMH) burdens. Patients with higher WMH burden were more prone to have higher median number of involved arteries.

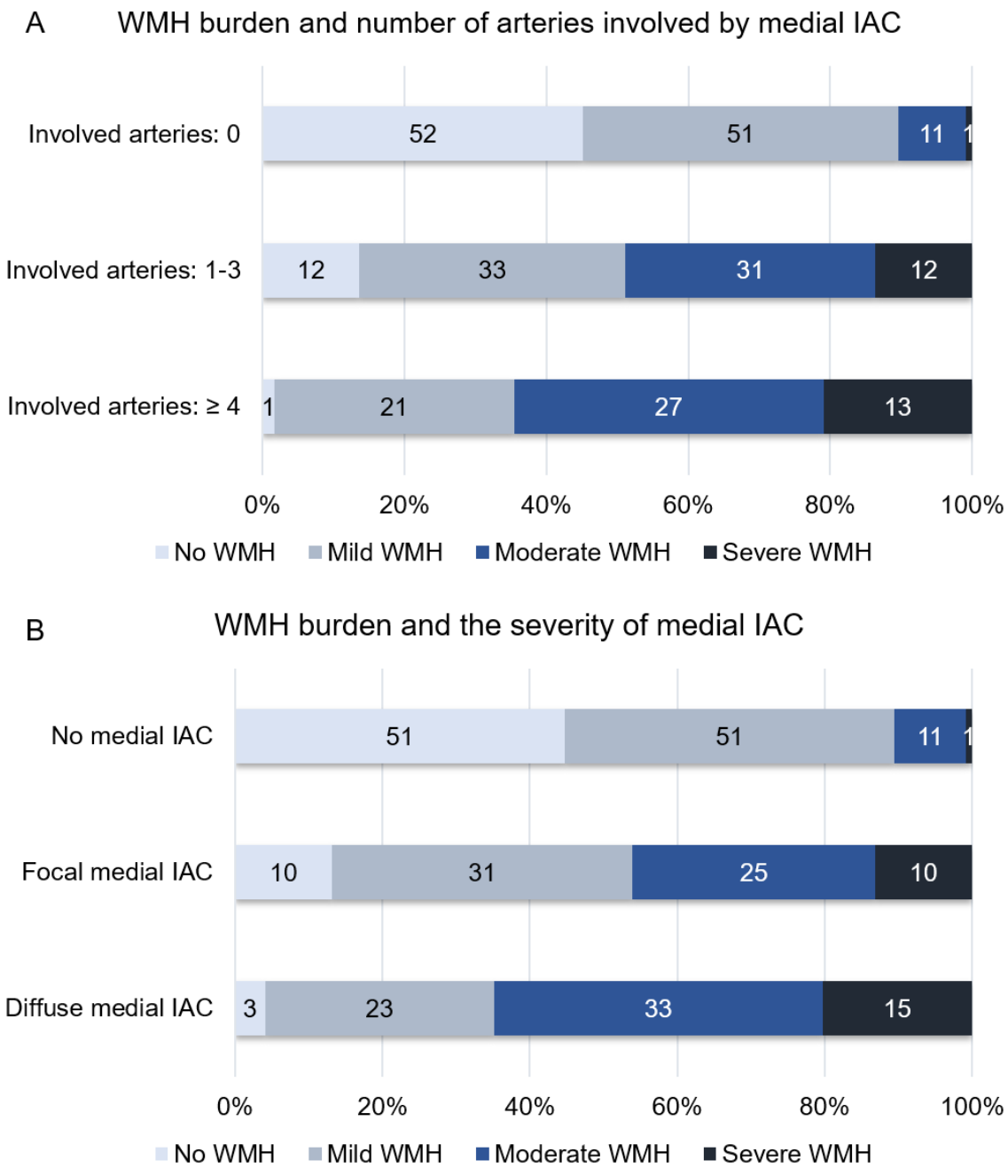

Figure 4-6. Correlation between the number of arteries involved buy medial intracranial arterial calcification (medial IAC) and the severity of medial IAC and the burden of white matter hyperintensities (WMH). Patients with more arteries involved by medial IAC (A) or more severe medial IAC (B) were more prone to have high WMH burden.

Binary logistic regression (**Table 4-3**) suggested that the presence of medial IAC was correlated with the presence of WMH (crude OR, 8.698; 95% CI, 4.420-17.119; $p<0.001$). After adjusting for confounding factors (age, hypertension, previous stroke/TIA and ischemic heart disease), multiple binary logistic regression indicated that the correlation remained statistically significant (adjusted OR, 3.794; 95% CI, 1.741-8.267; $p<0.001$).

The correlation between WMH burden and the presence, the count and the involvement of medial IAC were examined by ordinal logistic regression models (**Table 4-4**). The presence of medial IAC suggested higher risk of higher WMH burden, before (crude OR, 9.839; 95% CI, 5.719-16.629; $p<0.001$) and after (adjusted OR, 4.947; 95% CI, 2.780-8.801; $p<0.001$) adjusting for confounding factors. Compared with the absence of medial IAC, 1 to 3 arteries involved by medial IAC (adjusted OR, 3.956; 95% CI, 2.141-7.310; $p<0.001$) and 4 or more arteries involved by medial IAC (adjusted OR, 7.215; 95% CI, 3.549-14.667; $p<0.001$) indicated higher risk of high WMH burden, after adjusting for confounding factors. Similarly, compared with the absence of medial IAC, focal medial IAC (adjusted OR, 3.966; 95% CI, 2.107-7.466; $p<0.001$) and diffuse medial IAC (adjusted OR, 6.506; 95% CI, 3.297-12.841; $p<0.001$) were also correlated with risk of high WMH burden, after adjusting for confounders.

Table 4-3. **Multiple binary logistic regression on the presence of medial intracranial arterial calcification (IAC) and the occurrence of white matter hyperintensities (WMH).**

	WMH occurrence					
	Crude OR (95% CI)	***p* value**	**Model-1 OR (95% CI)**	***p* value**	**Model-2 OR (95% CI)**	***p* value**
Medial IAC presence	8.698 (4.420-17.119)	<0.001*	4.011 (1.878-8.567)	<0.001*	3.794 (1.741-8.267)	<0.001*

Model-1: adjusted for age and hypertension. Model-2: adjusted for age and hypertension, previous stroke/TIA and ischemic heart disease. OR, odds ratio. CI, confidence interval. *: $p<0.05$

Table 4-4. Multiple ordinal logistic regression models about the correlations between medial intracranial arterial calcification (IAC) and white matter hyperintensities (WMH).

	WMH burden (absent, mild, moderate and severe)					
	Crude OR (95% CI)	***p* value**	**Model-1 OR (95% CI)**	***p* value**	**Model-2 OR (95% CI)**	***p* value**
Medial IAC, presence						
Absent	1.000		1.000		1.000	
Present	9.839 (5.719-16.629)	<0.001*	5.075 (2.861-9.002)	<0.001*	4.947 (2.780-8.801)	<0.001*
Medial IAC, involved arteries						
0 arteries	1.000		1.000		1.000	
1 to 3 arteries	7.362 (4.101-13.214)	<0.001*	4.081 (2.215-7.520)	<0.001*	3.956 (2.141-7.310)	<0.001*
≥ 4 arteries	15.295 (7.852-29.792)	<0.001*	7.365 (3.638-14.911)	<0.001*	7.215 (3.549-14.667)	<0.001*
Medial IAC, severity						
No medial IACs	1.000		1.000		1.000	
Focal medial IAC	6.851 (3.751-12.512)	<0.001*	4.131 (2.207-7.732)	<0.001*	3.966 (2.107-7.466)	<0.001*
Diffuse medial IAC	14.887 (7.852-28.227)	<0.001*	6.589 (3.342-12.989)	<0.001*	6.506 (3.297-12.841)	<0.001*

Model-1: adjusted for age and hypertension. Model-2: adjusted for age and hypertension, previous stroke/TIA and ischemic heart disease. OR, odds ratio. CI, confidence interval. *: $p<0.05$

4.3.3 Interrater reliability of assessments on IAC and WMH

The inter-rater reliabilities of IAC presence, IAC pattern (intimal and medial) and IAC involvement (focal and diffuse) were evaluated separately. The weighted kappa of IAC presence was 0.904 (95% CI, 0.839-0.969, p<0.001), the weighted kappa of IAC pattern was 0.877 (95% CI, 0.760-0.993, p<0.001) and the weighted kappa of IAC severity was 0.871 (95% CI, 0.747-0.995, p<0.001). WMH presence and WMH burden were also evaluated separately. The weighted kappa of WMH presence was 0.932 (95% CI, 0.802-1.063, p<0.001) and the weighted kappa of WMH burden was 0.860 (95% CI, 0.730-0.990, p<0.001).

4.4. Discussion

In summary, this study for the first time identified that the count and the involvement of medial IAC were independently associated with the presence and burden of WMH while intimal IAC did not correlate to WMH. With the count and involvement of medial IAC being higher, the burden of WMH was heavier. These findings may suggest that medial pattern of calcification in intracranial vascular beds may be a potential biomarker for CSVD.

The inherent difference between intimal IAC and medial IAC may be correlated with different vascular consequences. Intimal IAC is associated with large artery atherosclerosis[8,9] while medial IAC is more related to arterial stiffness[71,161], which may cause impaired vasodilation and vessel compliance. Although several studies suggested possible correlations between intracranial atherosclerosis and WMH[164-166], in consideration of distinct etiology of large artery atherosclerosis and CSVD, such correlations are more likely explained by mutual risk factors such as hypertension and aging[6,167]. CSVDs are mainly systemic vascular disorders and may occur without hypertension[168], suggesting more complex pathogenesis than arteriolar occlusions. In ischemia led by CSVD, restricted vessels may lead to a state of chronic

hypoperfusion in the microvascular beds that supplies the white matter. Eventually, the myelinated fibers degenerate as a result of repeated oligodendrocyte damage[169]. One of the causative roles in white matter lesions may be altered intracranial vascular pulsatility[170], which could result from the progressing arterial stiffness. As medial IAC evolves widely in multiple intracranial vessel beds, the degree of global arterial stiffness may advance, thus leading to restricted vasodilation and hypoperfusion in vast subcortical regions during needs of increased neuronal activity and metabolic or vasodilatory challenge. On the other hand, increased pulsatile wave transmission from the aorta to the microcirculation caused by arterial stiffness may also be hazardous to cerebral microvasculature[171]. In line with our findings, a recent study identified that the volume of medial IAC, rather than that of intimal IAC, mediates the association between blood pressure and WMH[172]. These findings indicate that medial IAC, as a proxy for arterial stiffness, may play an independent role in the pathogenesis of CSVD. Given the fact that evaluation of subcortical cerebrovascular activity is difficult, our results may benefit the understanding of CSVD from a different aspect.

The findings of this study indicate the relationship between medial IAC and WMH burden. Although the investigation was based on stroke patients, it could still be hypothesized that medial IAC may be a potential marker for the emergence of WMH in general population. Cerebral small vessel diseases and IAC have shared risk factors, such as aging, diabetes and hypertension. The detection of severe medial IAC may indicate the burden of WMH and enhance the necessity of a more comprehensive assessment on patients with risk factors. On the other hand, the dose-effective relationship between medial IAC and WMH suggests that medial IAC may affect the development of WMH. Due to artery stiffening led by medial IAC, the competence of cerebrovascular autoregulation system would be limited when responding to hypoperfusion and new collaterals, which might be hazardous to cerebral microvasculature. Future investigations may unravel more detailed mechanisms between the evolvement of IAC

and WMH, providing evidence for more delicate managements of blood pressure and other risk factors of strokes and thereby contributing to the prevention of stroke.

Associations between IAC and cognitive disorders were reported. Larger IAC volume was found to be correlated with worse cognitive performance[139] and higher risk of cognitive decline[138]. Cognitive impairment was found to be associated with IAC in patients with chronic kidney disease that may cause irregular serum calcium[137]. However, despite the high prevalence of IAC in patients with cognitive disorders, IAC may not be directly related to cognitive outcome[173,174]. The bridge that connects IAC with cognitive impairment could be CSVD. White matter lesions led by CSVD have been found to be a predictor of cognitive decline[175] and dementia[176]. By far, neuroimaging has revealed notable association between WMH and cognitive impairment[177,178]. The total WMH volume is a strong predictor of cognitive impairment[179]. Increasing WMH burden is correlated with diffuse loss of cortex and white matter in vast brain areas, resulting in reduced global network efficiency[168] and worse cognitive performance[180]. Moreover, WMH also have impact on movement disorder[181] and neuropsychiatric symptoms[182] in people with cognitive disorders. By identifying medial IAC as a potential biomarker for CSVD, this study could provide new perspectives about the correlation between IAC and cognitive impairment in future studies.

Identifying medial IAC as an imaging biomarker of CSVD may also contribute to the determination of potential candidates for additional MRI scanning among admitted patients. CSVD accounts for nearly half of all dementias[168], of which the economic burden is high. In China, the annual total national cost of dementia ranges from $ 69 billion to $ 195 billion[183,184] and the expense per patient tends to rise with the progression of dementia[185]. Considering population aging and the increasing prevalence of dementia, early detection of CSVD and CSVD-related dementia is essential for reducing costs. The symptoms and clinical course of CSVD are highly variable[168], hence medial IAC, as a potential imaging biomarker, may be

helpful during patient screening. In real-world clinical activities, medical supplies, especially imaging assessments, are invariably inadequate to fully cover every aspect for individuals. As a result, studies on the cost-effectiveness of brain MRI modality for patients with potential CSVD may be needed in the future. It has been reported that in patients with minor stroke, additional brain MRI scanning has been found to enable timely secondary prophylaxis, which results in lower costs and higher cumulative quality-adjusted life years[186]. In light of the heavy burden of CSVD-related functional disability and cognitive impairment, appropriate brain MRI scanning will most likely ensure early detection and timely interventions on CSVD, thereby reducing long-term expense and improving final outcomes.

Renal impairment was previously reported to be correlated with WMH. Peri-ventricular WMH might be correlated with renal impairment among people under 60-year-old, but the correlation was found to be attenuated in population with age over 60 years[187]. Stroke survivors with a creatinine clearance level < 60ml/min may have larger volume of WMH, compared to counterparts with creatinine clearance > 60ml/min[188,189]. Considering the findings in these studies, we assessed the relationship between WMH and kidney function in our cohort but no significant associations were identified in our analysis.

There several limitations in this study. The first is the small sample size. The relatively small proportion of severe WMH in all WMH categories may impose restrictions upon the effectiveness of ordinal regression analysis, by limiting the degrees of freedom and adjustable confounders. As a result, unavoidable overestimations may dwell in the identified relationships between WMH and medial IAC. Secondly, the patients in this cohort were recruited from single stroke center. The prevalence of IAC and WMH burden may be different on a larger scale, which could lead to different discoveries when assessing the correlations between WMH and IAC. Since the cohort consisted of stroke patients, the proportions of IAC and WMH were undoubtedly higher than that among general populations. Additionally, there may be a mortality

or selection bias due to the fact that more severe patients are less likely to undergo MRI. The interpretations on the relationship between WMH and IAC that were generated from this study may be limited. Thirdly, this study was a cross-sectional study with no longitudinal observations. In this present study, we identified a dose-effective relationship between medial IAC and WMH burden. Although it is reasonable to assume that IAC, as part of the systematic calcification process occurring in all vessel beds, might not be the consequence of vessel disorder located merely in brain, we lack evidence that leads to the causality between IAC and WMH. The mechanisms remain unrevealed at the moment. Moreover, in this present study, the cognitive status of the patients was not included, which may also increase the clinical applicability of interpreting the correlations between small vessel disease and medial IAC. Future clinical and basic studies may be needed to identify the impact of medial IAC on cerebral small vessel diseases.

4.5. Conclusions

The overall amount and local involvement of medial pattern of IAC are associated with the presence of WMH. With intracranial vessel beds being more widely involved by medial IAC, the burden of WMH is heavier. These findings indicate a potential impact of medial IAC related arterial stiffness on the pathogenesis of cerebral small vessel disease. Future studies may be needed to focus on the role of medial IAC in regulating cerebral hemodynamics and microcirculation.

Chapter 5. Substudy-3: The Effect of Intracranial Arterial Calcification on Reperfusion Therapy in Acute Stroke Patients: A Cohort Study

Chapter 5. Substudy-3

5.1 Background

Intracranial arterial calcification (IAC) has been suggested to be an important imaging marker to predict ischemic stroke and guide clinical management of stroke patients[11,12]. Higher IAC volume was found to be associated with incomplete arterial revascularization after endovascular thrombectomy (EVT)[18] and hemorrhagic transformation after intravenous thrombolysis (IVT)[130]. Stroke patients with higher IAC burden (number of calcified major intracranial arteries) were reported to experience higher rates of vascular events[17] and poorer functional outcome[116]. While most of the previous studies worked on the volume and the burden of IAC, the pattern of IAC has not been fully studied yet. IAC can be classified as intimal and medial pattern based on its location in arterial wall[8]. Increasing histological and imaging studies suggested that intimal IAC is closely related to atherosclerosis[8,9] while medial IAC leads to arterial stiffness[161]. A recent study based on endovascular thrombectomy (EVT) identified that with the presence of medial IAC rather than intimal IAC in the internal carotid artery, patients may benefit in 90-day functional outcome after EVT, compared to patients who did not undergo EVT[16]. Accordingly, we hypothesized that among acute stroke patients, IAC patterns are associated with the clinical therapeutic effect on neurologic function recovery in patients who receive reperfusion therapy (IVT and bridging therapy).

In this study, we aimed to investigate the effects of IAC patterns on the outcome of acute stroke patients receiving reperfusion therapy.

5.2 Materials and methods

5.2.1 Subjects

The present prospective patient cohort study was approved by the Clinical Research Ethics Committee of the Peking University Shenzhen Hospital. Consecutive patients admitted

to the stroke center from 2020 to 2021 were recruited. The inclusion criteria were as follows: 1) patients above 18 years old who were diagnosed as acute cerebral infarction; 2) reperfusion therapies were conducted, including IVT and bridging therapy (IVT with subsequent EVT). IVT was performed within 4.5 hours after stroke onset, with or without following endovascular thrombectomy (EVT) within 6 hours based on local guidelines and the evaluations of clinical neurologists; 3) non-contrast brain computed tomography (CT) was performed before reperfusion therapy. Follow-up brain CT was performed after reperfusion therapy in patients with suspected intracerebral hemorrhage. The exclusion criteria were as follows: 1) contraindications to reperfusion therapy; 2) baseline modified Ranking Scale (mRS) score ≥ 2; 3) poor quality of imaging data or incomplete clinical data.

5.2.2 Clinical assessments

Baseline characteristics including gender, age, and vascular risk factors such as hypertension, diabetes and atrial fibrillation were recorded. Smoking was defined as current smoker and drinking was defined as having daily consumption of alcohol. The size of ischemic stroke lesion was assessed by The Alberta Stroke Program Early CT score (ASPECTS) in patients with anterior circulation stroke and Posterior Circulation ASPECTS (pc-ASPECTS) in patients with posterior circulation stroke. The severity of neurological dysfunction was assessed using the National Institute of Health stroke scale (NIHSS). The primary outcome of this study was favorable neurologic outcome (FNO), defined as either NIHSS reduction ≥ 8 points or final NIHSS ≤ 3 on within 10 days after reperfusion therapy[190,191]. The secondary outcome was early neurologic deterioration (END), defined as an increase of NIHSS ≥ 4 points, explainable by stroke compared to the post-therapy NIHSS, or death within 10 days after reperfusion therapy[192]. END includes stroke progression, stroke recurrence, symptomatic

cerebral hemorrhage and death[193,194]. Stroke progression was defined as worsened stroke without intracerebral hemorrhage or stroke recurrence[192-194]. Stroke recurrence was defined as a sudden, persist dysfunction after therapy affecting an artery territory that was different from the original stroke territory[194]. Symptomatic cerebral hemorrhage was defined as intraparenchymal hemorrhage identified on brain CT[195].

5.2.3 Evaluation on intracranial arterial calcification

Intracranial arterial calcification (IAC) was identified on CT images before the procedure of reperfusion therapy. Major intracranial arterial segments were examined, including C3 to C7 segments of the internal carotid artery (ICA), M1 segment of the middle cerebral artery (MCA), V4 segment of the vertebral artery (VA) and the basilar artery (BA). The presence of IAC was defined as hyperdense foci over 130 Hounsfield units. IAC was measured by two independent neurologists (H.D. and X.L) who were blinded to clinical characteristics. IAC was classified into two pathologic patterns: intimal IAC and medial IAC, based on a previously developed and validated grading scale[21]. The grading scale assessed circularity (1 point for dot, 2 for <90 degrees, 3 for 90-270 degrees and 4 for 270-360 degrees), thickness (1 point for thick IAC ≥ 1.5mm and 3 for thin IAC < 1.5mm) and morphology (0 point for indistinguishable, 1 for irregular/patchy and 4 for continuous) of each IAC. A summed score ranging from 1 to 6 indicated predominant intimal IAC and a score from 7 to 11 was considered as medial IAC *(***Figure 5-1**). In patients with IAC, calcification was further categorized based on the involvement, which was defined by the trajectory along intracranial arteries: 1) diffuse IAC was calcification of which the trajectory extended over 1/3 of the examined intracranial arteries and 2) focal IAC was defined as the trajectory of IAC involving less than 1/3 of the examined intracranial arteries (**Figure 5-2**). Additionally, single diffuse

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IAC was defined as only one intracranial artery segment involved by diffuse IAC and multiple diffuse IAC was defined as two or more examined intracranial arteries involved by diffuse IAC.

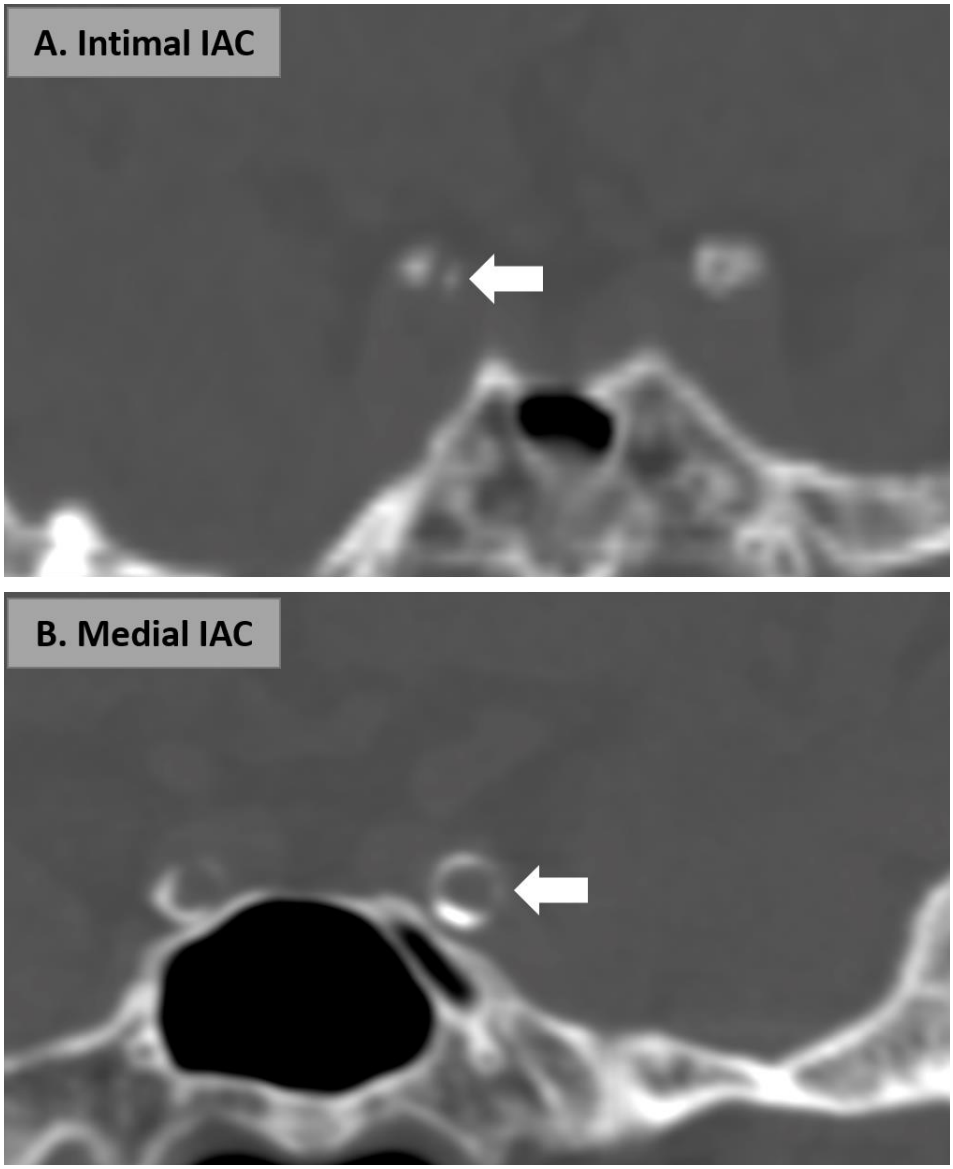

Figure 5-1. Intracranial arterial calcification (IAC) patterns on brain computed tomography (CT) images: intimal IAC (A) and medial IAC (B).

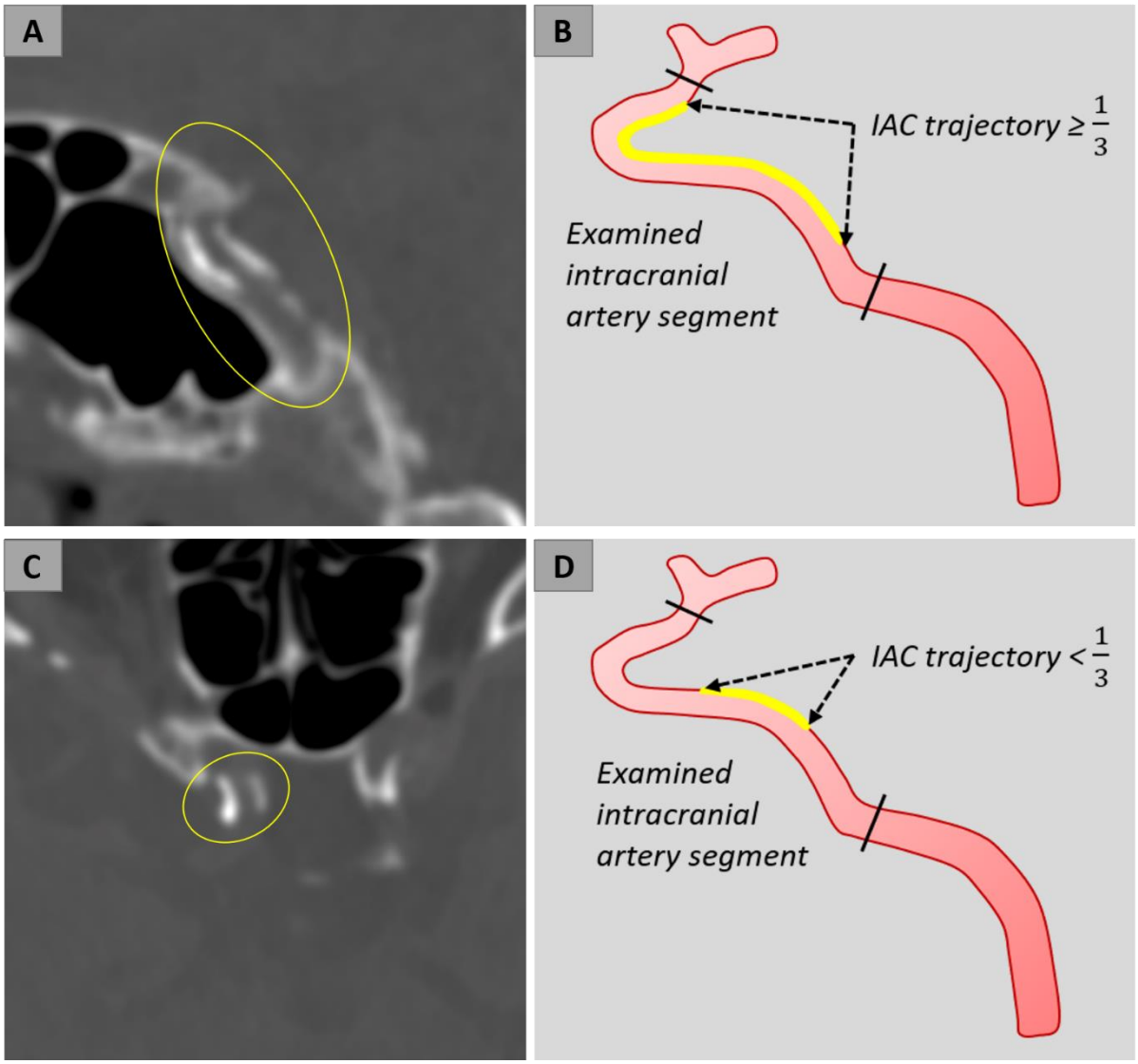

Figure 5-2. Diffuse IAC (A) extended over 1/3 of the length of the examined intracranial artery segment (B) and focal IAC (C) involved less than 1/3 of the examined intracranial artery segment (D).

5.2.4 Statistical Analysis

IBM SPSS (20.0, SPSS, Inc) was used for statistical analysis. Continuous variables were expressed by mean ± standard deviation (SD) and categorical variables were presented as numbers and percentages. The inter-rater reliability of IAC measurement was assessed by Cohen's kappa analysis. Independent T-test was used for comparisons of age and NIHSS score between different IAC groups (intimal IAC vs. medial IAC; diffuse IAC vs. focal IAC). Pearson's chi-square test or Fisher's exact test was used for comparisons of other risk factors, FNO and END between different IAC groups. In the identification of confounding factors, the association between age and FNO or END was based on independent T-tests; the association between other risk factors and FNO or END was based on Pearson's chi-square test or Fisher's exact test. Factors with p-value less than 0.1 were considered as confounding factors. Multiple linear regression was used for examining the correlation between NIHSS and different patterns of IAC. Multiple binary logistic regression was used to examine the correlation between different patterns of IAC and FNO or END.

5.3 Results

Of the 135 patients that met inclusion criteria, 5 were excluded because of incomplete scans (n=4) and unavailable clinical data (n=1). Among all the 130 included patients (mean age ± SD, 64.62 ± 13.69 years old) in this study, 81 (62.3%) were male. Thirty-six patients (27.7%) had diabetes, 78 (60.0%) patients had hypertension, 17 (13.1%) patients had hyperlipidemia and 21 (16.2%) patients had atrial fibrillation. Previous history of stroke or TIA was reported in 43 (33.1%) patients. A history of ischemic heart disease was reported in 23 (17.7%) patients, current smoking in 47 (36.2%) patients, and drinking in 38 (29.2%) patients. Among the 130 stroke patients, 98 (75.4%) had ischemic stroke in the anterior circulation and 32 (24.6%) had

posterior circulation strokes. During the treatment procedure (IVT, IVT plus EVT), no patients received general anesthesia or sedation.

Intracranial arterial calcification was identified in 117 (90.0%) patients, with intimal and medial IAC identified in 97 (74.6%) and 84 (64.6%) patients, respectively. Coexisting IACs (intimal IAC and medial IAC simultaneously) were identified in 64 (54.7%) patients. Of the 117 patients with IAC, 43 (36.8%) had focal IAC and 41 (34.5%) had diffuse IAC. In patients with diffuse IAC, 25 had multiple diffuse IAC. All diffuse IACs were found to be medial IAC. Risk factors (**Table 5-1**) of different patterns of IAC and the identification of confounding factors **(Table 5-2**) were shown in tables below.

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Table 5-1. Comparisons of baseline characteristics between different patterns of intracranial arterial calcification (IAC).

Characteristics	Intimal IAC			Medial IAC			Diffuse/focal IAC		
	Absent (n=33)	Present (n=97)	*p* value	Absent (n=46)	Present (n=84)	*p* value	Focal IAC (n=76)	Diffuse IAC (n=41)	*p* value
Male sex, n (%)	22 (66.7)	59 (60.8)	0.550	32 (69.6)	49 (58.3)	0.206	48 (63.2)	24 (58.5)	0.624
Age, mean ± SD	60.67 ± 16.89	65.97 ± 12.22	0.054	54.26 ± 12.00	70.30 ± 10.99	<0.001	62.36 ± 11.75	74.12 ± 9.51	0.001
Smoking, n (%)	11 (33.3)	36 (37.1)	0.696	27 (58.7)	20 (23.8)	<0.001	31 (40.8)	10 (24.4)	0.076
Drinking, n (%)	11 (33.3)	27 (28.8)	0.549	18 (39.1)	20 (23.8)	0.066	26 (34.2)	9 (22.0)	0.167
Diabetes, n (%)	6 (18.2)	30 (30.9)	0.158	10 (21.7)	26 (31.0)	0.262	22 (28.9)	13 (31.7)	0.756
Hypertension, n (%)	16 (48.5)	62 (63.9)	0.118	18 (39.1)	60 (71.4)	<0.001	46 (60.5)	30 (73.2)	0.171
Hyperlipidemia, n (%)	4 (12.1)	13 (13.4)	1.00	5 (10.9)	12 (14.3)	0.581	14 (18.4)	2 (4.9)	0.042

Atrial fibrillation, n (%)	8 (24.2)	13 (13.4)	0.144	4 (8.7)	17 (20.2)	0.087	9 (11.8)	11 (26.8)	0.040
History of stroke or TIA, n (%)	9 (27.3)	34 (35.1)	0.412	9 (19.6)	34 (40.5)	0.015	24 (31.6)	17 (41.5)	0.285
History of ischemic heart disease, n (%)	7 (21.2)	16 (16.5)	0.540	4 (8.7)	19 (22.6)	0.047	11 (14.5)	11 (26.8)	0.103

SD, standard deviation; TIA, transient ischemic attack.

Table 5-2. Comparisons of vascular risk factors in favorable neurologic outcome (FNO) and in early neurological deterioration (END).

	FNO			END		
	No (n=47)	Yes (n=83)	*p* value	No (n=108)	Yes (n=22)	*p* value
Male sex, n (%)	26 (55.3)	55 (66.3)	0.216	69 (63.9)	12 (54.5)	0.410
Age, mean ± SD	68.60 ± 12.88	62.37 ± 13.69	0.012	67.27 ± 11.85	64.08 ± 14.01	0.321
Smoking, n (%)	15 (31.9)	32 (38.6)	0.449	37 (34.3)	10 (45.5)	0.319
Drinking, n (%)	11 (23.4)	27 (32.5)	0.772	30 (27.8)	8 (36.4)	0.420
Diabetes, n (%)	15 (31.9)	21 (25.3)	0.418	29 (26.9)	7 (31.8)	0.635
Hypertension, n (%)	29 (61.7)	49 (59.0)	0.766	64 (59.3)	14 (63.6)	0.702
Hyperlipidemia, n (%)	7 (14.9)	10 (12.0)	0.644	13 (12.0)	4 (18.2)	0.666
Atrial fibrillation, n (%)	12 (25.5)	9 (10.8)	0.029	16 (14.8)	5 (22.7)	0.358
History of stroke or TIA, n (%)	15 (31.9)	28 (33.7)	0.832	36 (33.3)	7 (31.8)	0.890

History of ischemic heart disease, n (%)	12 (25.5)	11 (13.3)	0.078	19 (17.6)	4 (18.2)	1.000
Stroke territory						
Anterior circulation stroke, n (%)	39 (83.0)	59 (71.1)	0.130	77 (71.3)	21 (95.5)	0.017
Posterior circulation stroke, n (%)	8 (17.0)	24 (28.9)		31 (28.7)	1 (4.5)	
ASPECTS score						
ASPECTS, mean ± SD	9.46 ± 0.72	9.61 ± 0.79	0.140	9.57 ± 0.70	9.48 ± 0.98	0.878
pc-ASPECTS, mean ± SD	9.50 ± 0.76	9.54 ± 0.93	0.654	9.53 ± 0.88	9.00 ± 0.00	0.438

SD, standard deviation; TIA, transient ischemic attack; ASPECTS, The Alberta Stroke Program Early CT score; pc-ASPECTS, Posterior Circulation ASPECTS.

5.3.1 IAC and baseline NIHSS

No significant correlation was found by independent t-tests between baseline NIHSS and the presence of intimal IAC or medial IAC. In patients with IAC, baseline NIHSS was significantly higher in patients with diffuse IAC than in patients with focal IAC (9.54 ± 8.21 vs. 5.67 ± 4.79, p=0.011) (**Table 5-3**). Patients with multiple diffuse IAC had even higher baseline NIHSS compared to patients with focal or single diffuse IAC (12.04 ± 8.76 vs. 5.66 ± 4.94, p=0.002). After adjusting for confounding factors, multiple linear regression suggested that multiple diffuse IAC was correlated with higher baseline NIHSS (B coefficient 4.554; 95% CI, 1.859-7.250, p<0.001).

Table 5-3. Comparison of the baseline National Institute of Health Stroke Scale (NIHSS) between different patterns of intracranial arterial calcification (IAC).

IAC		Baseline NIHSS, mean ± SD	*p* value
Intimal IAC	Presence	6.55 ± 6.45	0.182
	Absence	8.33 ± 7.07	
Medial IAC	Presence	7.56 ± 6.92	0.195
	Absence	5.98 ± 6.01	
Diffuse IAC		9.54 ± 8.21	0.011*
Focal IAC		5.67 ± 4.79	
Multiple diffuse IAC		12.04 ± 8.76	0.002*
Focal or single diffuse IAC		5.66 ± 4.94	

SD, standard deviation;

* $p<0.05$

5.3.2 IAC and clinical outcome

For the 130 patients, 118 received IVT only and 12 patients underwent EVT bridging to IVT. After reperfusion therapy, 83 patients (63.8%) had FNO in the first 24 hours. The presence of medial IAC or intimal IAC was not significantly associated with FNO. In patients with IAC, diffuse IAC was found to be associated with FNO (p=0.047). Similar finding was identified in multiple diffuse IAC, compared to focal or single diffuse IAC (p=0.024) (**Table 5-4**). After adjusting for confounding factors, multiple binary logistic regression models (**Table 5-5**) suggested that multiple diffuse IAC was independently associated with FNO (OR 0.362; 95% CI, 0.146-0.893, p=0.027). Among the patients who received EVT, 4 had diffuse IAC and 8 had focal IAC. The occurrence of FNO was not observed after EVT in the 4 patients who had diffuse IAC. After excluding the 12 patients receiving IVT plus EVT, multiple binary logistic regression demonstrated that multiple diffuse IAC was correlated with less frequent FNO (adjusted OR, 0.398; 95% CI, 0.161-0.979). During the first 10 days after reperfusion therapy, 22 patients (16.9%) experienced END. Intimal IAC, medial IAC, and diffuse IAC were not found to be correlated with the occurrence of END.

Table 5-4. Comparison of effective favorable neurologic outcome (FNO) and early neurological deterioration (END) between different patterns of intracranial arterial calcification (IAC).

IAC		FNO			END		
		Yes (n=83)	No (n=47)	*p* value	Yes (n=22)	No (n=108)	*p* value
Intimal IAC	Presence, n (%)	63 (75.9)	34 (72.3)	0.654	16 (72.7)	79 (73.1)	0.968
Medial IAC	Presence, n (%)	49 (59.0)	35 (74.5)	0.077	15 (68.2)	69 (63.9)	0.701
Patients with IAC		Yes (n=74)	No (n=43)		Yes (n=20)	No (n=97)	
Diffuse IAC, n (%)		21 (28.4)	20 (46.5)	0.047*	8 (40.0)	33 (34.0)	0.610
Multiple diffuse IAC, n (%)		11 (14.9)	14 (32.6)	0.024*	4 (20.0)	21 (21.6)	1.00

SD, standard deviation; * $p<0.05$

Table 5-5. Multiple binary logistic regression on intracranial arterial calcification (IAC) and favorable neurologic outcome (FNO).

	Favorable neurologic outcome Odds ratio (95% Confidence interval)	*p* value
Crude association		
Diffuse IAC	0.456 (0.208-0.998)	0.049*
Multiple diffuse IAC	0.362 (0.146-0.893)	0.027*
Model-1: Adjusted for confounding factors (atrial fibrillation and history of ischemic heart disease)		
Diffuse IAC	0.519 (0.232-1.163)	0.111
Multiple diffuse IAC	0.362 (0.146-0.893)	0.027*
Model-2: Adjusted for confounding factors (age, atrial fibrillation and history of ischemic heart disease)		
Diffuse IAC	0.615 (0.253-1.491)	0.282
Multiple diffuse IAC	0.362 (0.146-0.893)	0.027*

* $p<0.05$

5.3.3 Interrater reliability of evaluations on IAC pattern

The inter-rater reliabilities of IAC presence, IAC pattern (intimal and medial) and IAC involvement (focal and diffuse) were evaluated separately. The weighted kappa of IAC presence was 0.904 (95% CI, 0.839-0.969, p<0.001), the weighted kappa of IAC pattern (intimal and medial) was 0.877 (95% CI, 0.760-0.993, p<0.001) and the weighted kappa of IAC involvement (diffuse and focal) was 0.871 (95% CI, 0.747-0.995, p<0.001).

5.4 Discussion

The present study demonstrated that compared to focal IAC, the diffuse pattern of IAC was correlated with higher baseline NIHSS, less favorable neurologic outcome in the acute stage of ischemic stroke after reperfusion therapy. Considering all diffuse IACs were found medial IAC, it suggested that a larger involvement of medial IAC may be associated with less favorable therapeutic effect of reperfusion therapy during the acute stage of ischemic stroke.

We identified that patients with a diffuse pattern of medial IAC had higher baseline NIHSS compared to patients focal IACs, suggesting a correlation between medial IAC and severe neurologic dysfunction. This may account for a larger infarct volume in 5 to 7 days after stroke onset reported in patients with medial IAC than those with intimal IAC[16]. Severe neurologic dysfunction may be due to arterial stiffening caused by medial IAC[196] which could result in impaired vascular compliance of the distal vessels[196,197]. A state of distal hypoperfusion might arise from the restriction of vessels, therefore leading to microvascular failure[169,198]. In fact, it has been reported that IAC may be correlated with imaging markers of small vessel disease, including white matter hyperintensities, lacunes and cerebral microbleeds[73,83,126]. In patients with small vessel occlusion, those had heavier IAC density were demonstrated to have higher risk of stroke recurrence[121]. These suggested the latent connection

between IAC and small vessel disorders.

This study firstly identified that a diffuse pattern of medial IAC was associated with less favorable neurologic outcome after reperfusion therapy in the acute stage, which was partly different from prior studies suggesting that patients with medial IAC might benefit from EVT[16] or IVT[93] in functional outcome. In fact, prior studies did not evaluate the involvement of IAC, compared to our present study. Intimal IAC commonly coexists with atherosclerosis that is known to cause endothelial damage[8,9,199-201]. As a consequence, the presence of intimal IAC might suggest a proneness of endothelial damage that is vulnerable to secondary injury after reperfusion. Compared to intimal IAC, medial IAC was reported in a previous study to be associated with better collateral status in patients who received IVT[93]. This may correlate to the association between medial IAC and better functional recovery in previous studies, as good collateral filling may predict successful reperfusion[40], favorable outcome and less recurrent strokes[37,39]. However, opposite findings were also reported in studies which included patients with EVT[16,202], suggesting poorer collateral circulation in patients with medial IAC. We hypothesize that the discrepancy might result from the unstratified involvement of medial IAC that account for potential vascular injuries. In contrast to intimal IAC that is related to large artery disease[8,9,203], medial IAC might be associated with cerebral small vessel disease. When a large intracranial artery is occluded, the blood supply tends to be more dependent on the capacity of microvascular beds than that in branch occlusion. Considering the function of small vessel be hypothetically interfered by vasodilation restriction due to elevated arterial stiffness, a poorer collateral status might be observed among patients with severe medial IAC in studies based on EVT. This could also explain the findings in our study: when medial IAC emerges initially as a focal lesion, it may not have the capacity to affect the overall cerebral vascular bed. As medial IAC evolves and turns into a diffuse pattern, the status of microvascular beds might be more significantly damaged, thus leading to worse neurologic outcome in ischemic

stroke.

One considerable novelty of this study is that we further categorized the patterns of IAC based on its degree of involvement in intracranial arteries. Calcification with long trajectory along the intracranial arteries is factually a common but unstudied phenomenon in clinical practice. It is notable that prior studies suggested that "severe" (larger volume, thickness or circumference) calcification in the intracranial ICA was related to incomplete arterial revascularization[18] and prolonged procedure time[115] in patients who had EVT. Since those two studies did not classify IAC pattern[18,115], it is conceivable that arterial stiffening led by severe medial IAC may play an important role in affecting the outcome of EVT. By identifying more severe neurological dysfunction in patients with diffuse medial IAC than in focal medial IAC, we could hypothesize that diffuse medial IAC is a notable biomarker of the severe global medial calcification in cerebral vessel beds. Apart from the possible damage of global medial IAC on microvascular beds, local vascular compliance may also influence the outcome of EVT. With a long trajectory of medial IAC, the compliance of the vessels may be limited, leading to abnormal vessel compliance or impeded pathway of the instruments led by extensive IAC. According to our results as well as other similar studies, we may speculate that severe arterial calcification, such as diffuse medial calcification, could lead to poor clinical outcome due to relatively low successful rate of thrombectomies. Due to the small number of patients receiving EVT plus IVT therapy in our study, we need to test our speculations by recruiting more stroke patients in our future study. Another novelty of the present study is the analysis on mixed IAC pattern. We found that mixed IAC pattern may predict better functional recovery for stroke patients, suggesting a possibility of benefit from reperfusion therapy in patients with complex vascular status (both atherosclerosis and arterial stiffening), which may be inconsistent with general consensus. These current findings in our study may provide new insights into risk stratification and clinical managements in ischemic stroke.

Chapter 5. Substudy-3

Arterial calcification is a process of osteochondrogenic transformation. During the formation of calcification, the architecture of the vascular wall may be distorted[67] and thereby causing vascular change, such as cerebral microbleeds[83]. Stroke patients with larger IAC volume were found more prone to have hemorrhagic transformation after IVT[130] and the presence of IAC may be related to intracerebral hematoma expansion[127]. Endothelial impairment led by IAC may account for the increased permeability of brain blood barrier[125], which lead to subsequent hemorrhagic events. Although we grouped cerebral hemorrhage with stroke progression and stroke recurrence, evidence from other studies might validate our findings, with no significant difference in the frequency of cerebral hemorrhage between different IAC patterns[16,93]. Another cohort-based study also documented similar frequencies of overall brain hemorrhage between patients with different IAC patterns[131]. In our study, IAC was categorized into pathological patterns while other studies focused on the volume or density. Future studies on the volume or density of specific IAC patterns might be needed for the correlation between IAC and hemorrhagic events.

This present study has limitations. First, in the present study, whether the neurologic outcome in the acute stage was associated with intermediate prognosis was not evaluated. It was reported that IAC pattern may be related to 90-day functional outcome. Investigating the correlation between early and intermediate clinical outcome and their correlation between diffuse IAC in future studies might provide further evidence for clinical practice, such as rehabilitations for disabled patients. Second, this is a cohort from single stroke center, the prevalence of IAC may be higher than that of general population and thereby limit the interpretation of our findings. Third, compared to previous studies with thousands of subjects, the cohort in this study was relatively small, which limited the degrees of freedom and adjustable confounders. In addition, limited by the imaging modalities of the hospital from which our cohort was built, we did not have further imaging data of CT perfusion, CT

angiography or digital subtraction angiography to assess the perfusion and collateral status, which may limit the interpretation of our findings. At last, most of the patients who had only IVT were not able to be examined by angiography before the treatment according to local guidelines. As a result, we lacked the information about intracranial large artery or medium artery occlusion before reperfusion therapy. Since the effect of reperfusion therapies is largely dependent on the presence of an arterial occlusion, this limitation may explain in part the lack of positive results observed in this investigation.

5.5. Conclusion

This present cohort-based prospective study identified a potential impact of IAC pattern on the acute clinical outcome of reperfusion therapy. Diffuse IAC is associated with more severe neurologic dysfunction and less favorable neurologic outcome from reperfusion therapy, suggesting this pattern of medial IAC may be a biomarker for clinical assessment and therapeutic strategies. Our findings may enrich the current understanding the impact of IAC pattern and provide insights into future clinical managements on ischemic stroke.

Chapter 6. Substudy-4: Effect of Intracranial Arterial Calcification on the Etiology of Acute Ischemic Stroke

Chapter 6. Substudy-4

6.1 Background

Intracranial arterial calcification (IAC) is an independent risk factor for ischemic stroke[11], which refers to divergent etiology of cerebral infarct, including large artery atherosclerosis, small vessel occlusion and cardioembolism[204]. Despite that IAC was found to be associated with the occurrence[11,12] and recurrence of stroke[120,121], previous studies mostly focused on the relation between IAC and strokes of all causes while the correlation between stroke etiology and IAC was less studied. Moreover, the difference between IAC embedded in different layers of artery wall was less investigated, either. There are two major pathological patterns of IAC: intimal and medial. Intimal IAC was more associated with the process of atherosclerosis[8,9], and medial IAC might play an important role in arterial stiffening[205]. The inherent pathologic difference between intimal IAC and medial IAC might result in different stroke etiology. By far, little has been studied on the correlation between IAC pattern (intimal and medial) and specific stroke etiology. In the present study, we aimed to investigate the correlation between IAC pattern and three major stroke etiology: small vessel occlusion, large artery atherosclerosis and cardioembolism.

6.2 Methods and materials

6.2.1 Subjects

This study was approved by the Clinical Research Ethics Committee of the Peking University Shenzhen Hospital. Consecutive patients admitted to the stroke centre from November 2020 to April 2022 were recruited retrospectively. The inclusion criteria were: 1) patients above 18 years old who had acute ischemic stroke; 2) non-contrast brain computed tomography (CT) and magnetic resonance imaging (MRI) were performed within 7 days after symptom onset; 3) the etiology of ischemic stroke was either large artery atherosclerosis,

cardioembolism or small vessel occlusion, according to the TOAST classification[204]. The exclusion criteria were as follows: 1) contraindications to CT or MRI; 2) patients with critical medical condition, including head trauma and brain tumor; 3) poor quality of imaging data and incomplete clinical data.

6.2.2 Imaging analysis and assessment on stroke etiology

The assessment of IAC was based on brain CT. Intracranial artery segments including the lacerum and cavernous segment (C3-C4) of internal carotid arteries (ICAs), the supraclinoid segment to the communicating segment (C5-C7) of ICAs, M1 segment of middle cerebral arteries (MCAs), proximal and distal part of the intracranial (V4) segment of vertebral arteries (VAs) and the basilar artery (BA) were examined. CT images were assessed by two neurologists with more than 5 years' experience of brain imaging (XL.L. and H.D.) who were blinded to clinical information and MRI images. The presence of IAC was defined as hyperdense foci over 130 Hounsfield units (HU). Based on a previously developed and validated grading scale[21], IAC was categorized into two patterns: intimal IAC and medial IAC (**Figure 6-1**). Circularity (1 for dot, 2 for < 90 degrees, 3 for 90-270 degrees and 4 for 270-360 degrees), thickness (1 for thick IAC ≥ 1.5 mm and 3 for thin IAC < 1.5 mm) and morphology (0 for indistinguishable, 1 for irregular/patchy and 4 for continuous) were assessed and graded. A summed score from 1 to 6 was defined as predominant intimal IAC and 7 to 11 was defined as medial IAC. The etiology of admission stroke was determined by clinical neurologist based on clinical information and brain MRI (**Figure 6-2**). Small vessel occlusion (SVO) was determined as single recent small subcortical infarcts (also commonly called lacunar strokes) of which the diameter of the infarct lesion was less than 20 mm[159]. Large artery atherosclerosis (LAA) was determined as cortical and/or subcortical infarcts presumably due to intracranial artery stenosis. Cardioembolism was determined as single or multiple cortical and/or

subcortical infarcts with confirmed cardiac source of embolism[204].

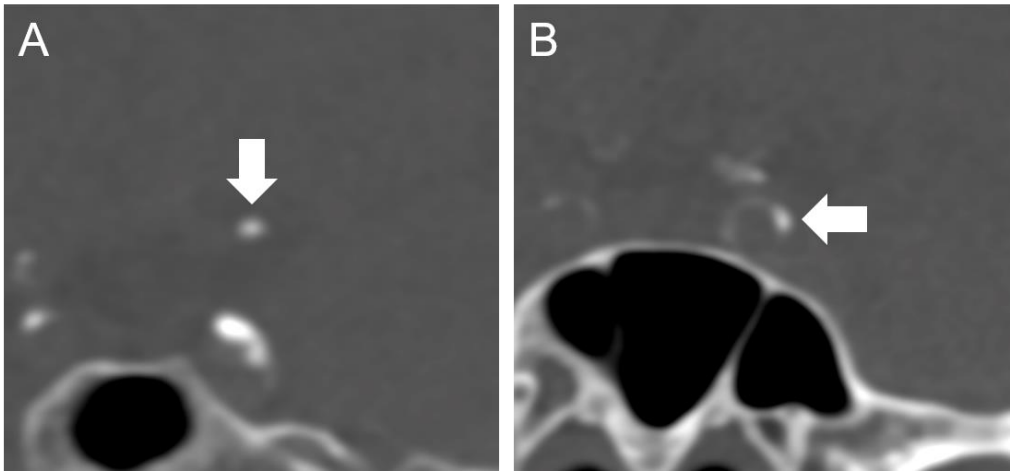

Figure 6-1. Intracranial arterial calcification (IAC) patterns on brain computed tomography (CT) images: intimal IAC (A) and medial IAC (B).

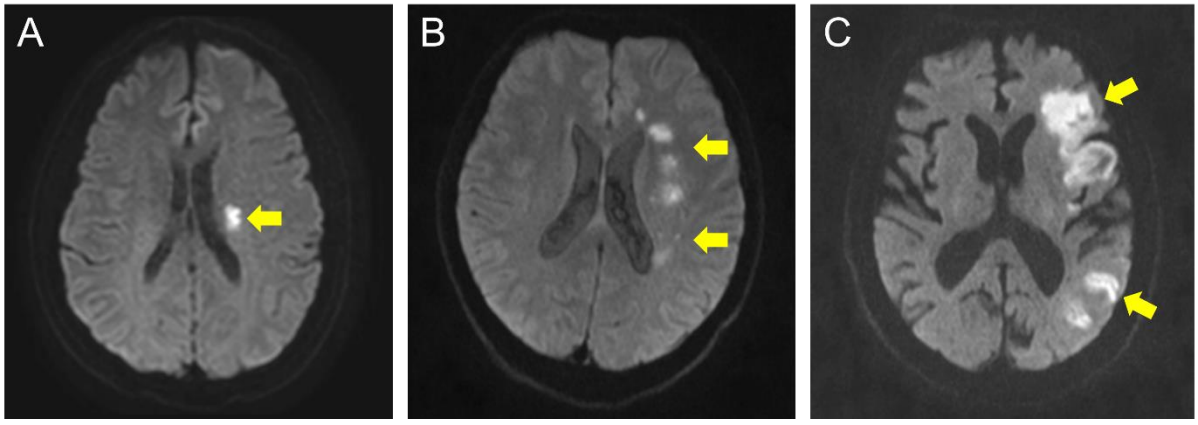

Figure 6-2. Different etiology of acute ischemic stroke: small vessel occlusion (A), large artery atherosclerosis (B) and embolism with determined cardiac source (C).

6.2.3 Statistic analysis

IBM SPSS (20.0, SPSS, Inc) was used for statistical analysis. Continuous variables were presented as mean ± standard deviation (SD) and categorical variables were presented as numbers and percentages. Cohen's kappa analysis was performed to examine the inter-rater reliability of IAC assessment. Independent t-test and Mann–Whitney U test were used for continuous variables. Pearson's chi-square test and Fisher's exact test were used for categorical variables. Vascular risk factors with p value < 0.1 or with high probable relation to stroke etiology were considered as confounding factors. Regression analysis was performed before and after adjusting for confounding factors. Multiple binary logistic regression analysis was performed to examine the correlations between IAC pattern and stroke etiology. A two-sided p less than 0.05 was regarded as statistically significant in the results.

6.3 Results

A total of 279 patients were included and 10 patients were excluded due to incomplete clinical data (3 patients) and poor image quality (7 patients). Among the 279 patients (mean age ± SD, 62.81 ± 12.14 years old), 187 (67.0%) were men. 67.0% patients had hypertension, 30.8% had diabetes and 18.3% had hyperlipidemia. Previous history of stroke or TIA was reported in 22.9% patients and history of ischemic heart disease was reported in 12.2% patients. Current smoking was reported in 41.6% patients and drinking was reported in 31.2% patients.

Intracranial arterial calcification was identified in 244 (87.5%) patients, with intimal IAC found in 178 (63.8%) patients and medial IAC found in 160 (57.3%) patients. Among all 279 patients, 52.0% patients had SVO, 41.2% patients had LAA and 6.8% were identified to have cardioembolism. Risk factors of different patterns of IAC were shown in **Table 6-1**. Chi-square tests showed no significant difference in stroke etiology between patients with intimal IAC and patients with medial IAC (**Table 6-2**). Univariate binary logistic regression analysis

also suggested that no statistically significant correlations were identified between stroke etiology and IAC pattern (**Table 6-3**).

The inter-rater reliabilities of IAC presence and IAC pattern were assessed separately. The weighted kappa of IAC presence was 0.904 (95% CI, 0.839-0.969, $p<0.001$) and the weighted kappa of IAC pattern was 0.877 (95% CI, 0.760-0.993, $p<0.001$).

Table 6-1. Baseline characteristics of patients with different stroke etiology.

Characteristics	SVO (n=145)	LAA (n=115)	Cardioembolism (n=19)	*p* value [a]	*p* value [b]	*p* value [c]
Male sex, n (%)	101 (69.7)	77 (67.0)	9 (47.4)	0.642	0.052	0.099
Age, mean ± SD	62.26 ± 11.79	62.24 ± 12.06	70.42 ± 13.31	0.990	0.006	0.008
Smoking, n (%)	60 (41.4)	51 (44.3)	5 (26.3)	0.631	0.207	0.140
Drinking, n (%)	49 (33.8)	34 (29.6)	4 (21.1)	0.468	0.264	0.446
Diabetes, n (%)	49 (33.8)	34 (29.6)	3 (15.8)	0.468	0.113	0.213
Hypertension, n (%)	101 (69.7)	72 (62.6)	14 (73.7)	0.232	0.718	0.351
Hyperlipidemia, n (%)	29 (20.0)	19 (16.5)	3 (15.8)	0.473	0.898	1.000
Previous stroke or TIA, n (%)	35 (24.1)	23 (20.0)	6 (31.6)	0.426	0.673	0.404
Ischemic heart disease, n (%)	15 (10.3)	11 (9.6)	8 (42.1)	0.835	<0.001	<0.001

SD, standard deviation; TIA, transient ischemic attack; SVO, small vessel occlusion; LAA, large artery atherosclerosis. [a]: SVO vs. LAA; [b]: SVO vs. Cardioembolism; [c]: LAA vs. Cardioembolism.

Table 6-2. Comparisons of intracranial arterial calcification (IAC) patterns in patients with different stroke etiology.

IAC pattern	SVO (n=145)	LAA (n=115)	Cardioembolism (n=19)	*p* value [a]	*p* value [b]	*p* value [c]
Intimal IAC, n (%)	87 (60.0)	80 (69.6)	11 (57.9)	0.110	0.860	0.313
Medial IAC, n (%)	82 (56.6)	64 (55.7)	14 (73.7)	0.885	0.154	0.140

SVO, small vessel occlusion; LAA, large artery atherosclerosis. [a]: SVO vs. LAA; [b]: SVO vs. Cardioembolism; [c]: LAA vs. Cardioembolism.

Table 6-3. Univariate binary logistic regression analysis on intracranial arterial calcification (IAC) and stroke etiology: small vessel occlusion (SVO), large artery atherosclerosis (LAA) and cardioembolism.

	Crude OR (95% CI)			*p* value [a]	*p* value [b]	*p* value [c]
Intimal IAC	1.524 (0.908-2.558)	0.917 (0.348-2.417)	0.602 (0.223-1.625)	0.111	0.860	0.316
Medial IAC	0.964 (0.589-1.579)	2.151 (0.736-6.288)	0.147 (0.754-6.606)	0.885	0.162	0.140

[a]: SVO vs. LAA; [b]: SVO vs. Cardioembolism; [c]: LAA vs. Cardioembolism. OR, odds ratio; CI, confidence interval.

6.4 Discussion

This study investigated the major etiology of acute ischemic stroke in patients with different IAC patterns. No significant correlation was found between IAC pattern and the etiology of acute ischemic stroke. The findings from this study suggested that the effect of IAC is less important on acute brain ischemia.

Vascular calcification is a chronic process of osteochondrogenic transformation of smooth muscle cells. During the process of atherosclerosis, intima becomes thickened while calcification forms gradually within the intima. After fusing into large clusters, intimal IAC may, under certain circumstances, ulcerate the intima and thereby resulting in thrombosis[205]. Although intimal IAC may be correlated with particular risky plaque features such as intraplaque hemorrhage[206], however, compared with the sophisticated mechanisms of atherosclerosis-related ischemic stroke, intimal IAC is merely a part of the whole pathogenetic process. Artery-to-artery embolism, hypoperfusion, branch artery occlusion all leads to brain ischemia[207], among which artery-to-artery embolism, hypoperfusion and their combination are the most common mechanisms of symptomatic intracranial atherosclerosis[105]. For instance, in patients with severe artery stenosis, hemodynamic deficiency that accounts for distal hypoperfusion is usually the dominant cause of brain ischemia, while IAC is less involved in the pathophysiologic process. On the other hand, apart from lipohyalinosis in small vessels, atherosclerotic plaque that infiltrate into the orifice of a penetrating artery also result in small subcortical infarct[207] which is often categorized as small vessel occlusion. Moreover, impaired clearance of emboli in patients with large artery occlusion may also cause small infarcts in the borderzone region[208], suggesting the difficulty of precise classification on stroke etiology. As a consequence, IAC may be less associated with the process of acute ischemic events.

Compared with acute ischemic stroke, chronic cerebral small vessel diseases may be more correlated with IAC. WMH and cerebral microbleeds were suggested previously to be

associated with IAC[83]. Recently, medial IAC volume was found to mediate the association between blood pressure and white matter hyperintensities[172], which provides new perspective on the correlation between cerebrovascular disease and IAC pattern. Cerebral small vessel diseases comprise of various pathophysiologic processes, such as arteriosclerosis, cerebral amyloid angiopathy, genetic abnormality, inflammatory and immunologically mediated small vessel disorders[169], of which arteriosclerosis may be the most related to IAC. The formation and extension of medial calcification may lead to artery stiffness. Vasodilation and compliance of the affected vessel would be damaged with the development of global artery stiffness, causing a chronic and persistent state of diffuse hypoperfusion in the microvascular beds. Therefore, the progress of arteriosclerosis might be accelerated by the evolving stiffness, which eventually lead to neurologic dysfunction, including cognitive impairment and mobility problems[168].

This study has several limitations. First, the sample size may not be large enough for particular etiology, e.g., cardioembolic strokes. Although the role of intra- and extra-cranial atherosclerosis on stroke are different between Asian and Caucasians (Asians more frequently have stroke due to intracranial atherosclerosis), the proportion of cardioembolic stroke in this study was still lower than expected. During clinical practice in the mainland, the patients are usually examined by regular echocardiography and Holter. As a result, there might be an undoubtable underestimation due to undetected cardiac source of embolism (e.g., patent foramen oval) and those patients would be probably considered to have “undetermined causes”. In this cohort, the proportion of cardiac embolism is 7.5%, which is lower but still briefly consistent with that in other centers in the mainland (11.7%; sample size: 623 patients; source: Beijing Tiantan Hospital)[209]. The design of this study is limited for sure, and we will seek to improve the workflow as much as we can in future studies. Second, this is a single-center study, so the prevalence of each etiology may be inconsistent with other centers. Third, due to the

lack of advanced imaging technique, especially vessel wall imaging using high resolution MRI, it was difficult to differentiate pure small vessel occlusion from branch occlusion caused by large artery atherosclerosis, which may lead to bias during etiology determination.

6.5 Conclusion

No significant correlations exist between IAC pattern and the etiology of ischemic stroke. The effect of IAC on acute symptomatic ischemic events might be less important. Studies with advanced imaging technique that are able to differentiate detailed mechanisms (e.g., artery-to-artery embolism, penetrating artery occlusion) may be needed in the future.

Chapter 7. Conclusions and Future Directions

Chapter 7. Conclusions and Future Directions

7.1 Conclusions

Since identified to be an independent risk factor for ischemic strokes[11], intracranial arterial calcification (IAC) has been drawing attention mostly as a proxy for intracranial atherosclerosis (ICAS) in clinical researches. However, even though calcification is prevalent in arteriosclerotic plaques that account for multiple mechanisms of ischemic stroke[6,105], there are still studies suggesting opposite roles of IAC[13,14]. Prior histopathologic evidence revealed that IAC not only exist under the surface of atheroma located at the intima, but also emerge in the medial layer of the vessel wall, of which the process is non-atherosclerotic[8,9]. There are mainly two patterns of IAC, the intimal pattern and the medial pattern. Intimal IAC is more correlated with progressive atherosclerotic plaques[8] that may lead to hemodynamic abnormalities while medial IAC is found to be more associated with artery stiffness[110]. Different patterns of IAC that form and evolve in separate vessel wall layers may lead to different pathophysiologic processes and divergent cerebrovascular consequences.

In the serial hospital-based studies, we explored the associations between IAC pattern and cerebrovascular diseases in the aspects of large arteriosclerosis, cerebral small vessel disease, reperfusion treatment and acute stroke etiology. In Chapter 3, we investigated pathology-validated imaging characteristics of intracranial large artery arteriosclerosis using high-resolution magnetic resonance imaging (HR-MRI) and assessed the associations between imaging characteristics and IAC pattern. The multimodal imaging-based study suggested that compared with medial IAC, intimal IAC more frequently coexists with luminal stenosis, eccentric plaque, higher plaque burden and intraplaque hemorrhage, which indicated a latent impact of intimal IAC on plaque vulnerability. The findings were also consistent with prior autopsies performed by our research team which demonstrated close associations between intimal IAC and ICAS.

Chapter 7. Conclusions and Future Directions

In Chapter 4, we investigated on the correlations between IAC pattern and white matter hyperintensities (WMH), one of the most common imaging-biomarkers for cerebral small vessel disease (CVSD). Different from large artery arteriosclerosis that often leads to acute neurological dysfunctions, CVSDs are more frequently associated with chronic neurological disorders, including cognitive impairment, dementia, depression and movement disorders[210]. The presence and burden of both IAC and WMH were assessed in the study. Medial IAC was found to be associated with the presence of WMH, and with intracranial vessel beds being more widely involved by medial IAC, the burden of WMH was heavier synchronously. Resulting from artery stiffness, altered intracranial vascular pulsatility may cause microvascular hypoperfusion and thereby white matter lesions. Considering the connection between artery stiffening and medial IAC, the findings may suggest potential impacts of medial IAC on the pathogenesis of CVSD.

In Chapter 5 and 6, we focused on the associations between IAC pattern and acute ischemic stroke, mainly on treatment and stroke etiology. In patients who received reperfusion treatment, a diffuse pattern of medial IAC was identified to be associated with more severe neurologic dysfunction and less favorable neurologic outcome from reperfusion therapy, suggesting this pattern of medial IAC may be a biomarker for clinical assessment and therapeutic strategies. The findings might be explained by the study in Chapter 4, which indicated potential correlations between medial IAC and small vessel disease. Patients with CVSD may have worse microvascular status, therefore having more severe neurologic dysfunction and less favorable outcome. On the other hand, IAC pattern was found to be less relevant to stroke etiology. It is known that acute ischemic strokes are attributed to multiple and sometimes mixed mechanisms. As a result, IAC may be merely part of the whole process of acute cerebral infarct. These findings provided perspectives for future studies on pathophysiologic associations between IAC and cerebrovascular diseases.

7.2 Future directions

7.2.1 The effect of IAC pattern on cerebral autoregulation

Cerebral autoregulation refers to the capability of intracranial vascular beds by means of sophisticated neurogenic and metabolic mechanisms to keep cerebral blood flow relatively constant over a wide range of blood pressure levels. During ischemic stroke, the function of cerebral autoregulation is crucial in the survival of penumbra, especially during interventions such as blood pressure management. Cerebral autoregulation is hypothesized to be impaired during chronic pathophysiologic processes (e.g. hypertension) that cause damage to arterioles and capillaries[211]. Based on the content of Chapter 4 in which the presence and the evolvement of medial IAC were found to be correlated with CVSD, it is probable that medial IAC is hazardous to the microvascular beds, thereby leading to autoregulation impairment. Moreover, since the cerebral blood flow is regulated by arterial blood pressure vascular conductance[212], artery stiffness led by medial IAC might also be a mediator that potentially accelerate the progress of autoregulation dysfunction. Further studies that investigate on the correlations between medial IAC and cerebral autoregulation are needed to answer theses questions and provide deeper understandings about the mechanisms of the whole process.

7.2.2 The impact of IAC pattern on endovascular revascularization treatment

In endovascular revascularization treatment, one of the concerns of operators is the safety of revascularization, especially during procedures like balloon dilatation and stent placing. The presence of complex calcified artery lesion would substantially elevate the level of brittleness of the vessel wall, leading to difficulty for instrument passes and increased risk of vessel rupture. As a result, proper choice of instruments, delicate expansion of balloon during

revascularization treatment would surely lower the risk of unexpected vessel rupture. In addition, during thrombectomy, the pathway of the extraction of thrombus may be blocked by large calcified lesions or stiffened vessel wall. As a consequence, the passes of retriever may increase, leading to prolonged procedure time and worse outcome. By further investigation on the potential impact of IAC pattern on endovascular revascularization, more information could be unraveled for better assessment before endovascular procedures.

7.3 Summary

Intracranial arterial calcification, as an independent risk factor for ischemic stroke, has gained increased attention from diagnosis to treatment. In this thesis, we mainly focused on the correlations between IAC pattern and cerebrovascular diseases. We investigated the difference between intimal IAC and medial IAC in their associations with large artery arteriosclerosis, small vessel disease, the etiology and reperfusion treatment of ischemic stroke in multimodal imaging-based studies. Future studies should direct more comprehensive investigations about the effect of particular IAC pattern on the pathophysiology of stroke. Multi-center collaborative research will enable better understandings on IAC-related cerebrovascular disorders and benefit individualized stroke management for more patients.

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