In Vitro Assessment of Histology Verified Intracranial Atherosclerotic Disease by 1.5T Magnetic Resonance Imaging
Concentric or Eccentric?

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Background and Purpose—Clinical trial studies show that plaque eccentricity (symmetry) is among the plaque features that have been associated with more frequent cerebrovascular events. Plaque eccentricity of intracranial atherosclerotic disease is unclear because of lacking of cerebral artery specimens.

Methods—1.5T magnetic resonance imaging was performed in the postmortem brains to scan the cross sections of middle cerebral artery. Plaque eccentricity of histology-verified middle cerebral artery atherosclerosis was calculated on T1-weighted fat-suppressed sequence.

Results—Validated by histology, concentric atherosclerotic plaques were identified in 46 middle cerebral arteries (63.9%) on magnetic resonance imaging and eccentric plaques in 26 arteries (26.1%). Eccentric plaques showed higher maximum wall thickness and lower minimum wall thickness than concentric plaques (both \( P<0.001 \)). Plaque burden and brain infarctions were similar between concentric and eccentric plaques.

Conclusions—Intracranial atherosclerosis presents as eccentric or concentric in geometry, which may be not linked to intracranial plaque risk. Further in vivo imaging studies are needed to identify morphological features of intracranial plaques and to verify its association with brain infarctions. (Stroke. 2016;47:527-530. DOI: 10.1161/STROKEAHA.115.011086.)

Key Words: high resolution magnetic resonance imaging ■ histology ■ intracranial atherosclerosis ■ plaque eccentricity

Intracranial atherosclerotic disease (ICAD), as a most common cause of ischemic stroke, has been drawing increased attentions of clinicians and researchers in the worldwide.\(^1\) Advanced imaging techniques, especially High Resolution Magnetic Resonance Imaging (HRMRI), could visualize the pathology of intracranial arterial diseases.\(^2\) However, current knowledge about histology of intracranial atherosclerosis lags behind the development of updated imaging modalities due to lacking of vessel specimens. Our previous post-mortem study showed that both luminal stenosis and percentage of lipid area or intraplaque neovasculature may play a key role in leading to ischemic stroke.\(^3\) Recent clinical trial studies on carotid arteries showed that, in addition to luminal stenosis, plaque eccentricity (symmetry) is among the plaque features associated with more frequent cerebrovascular events.\(^4\) The aim of the current study was to investigate the eccentricity of histology-verified intracranial atherosclerotic lesions on 1.5T MRI vessel wall imaging and its potential clinical implications.

Materials and Methods

Specimens

We recruited, consecutively, Chinese patients aged 45 years old or above, who died and underwent postmortem study at the Prince of Wales Hospital, Hong Kong (a regional general hospital) during a period of 19 months. The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

Among 58 elderly autopsy cases, 10 cases were excluded due to poor image quality (3 due to improper location of brain within the head coil, 3 due to inaccurate orientation of MRI scanning, 4 due to too large air rather than brain tissue which caused difficulties in acquiring proper imaging). Histology of middle cerebral arteries (MCAs) belonging to 10...
excluded autopsy cases was comparable to the recruited 48 cases. The median age of 48 recruited cases were 74 and male accounted for 55.3%. The incidence of risk factors, such as hypertension (42.6%), diabetes (27.7%), atrial fibrillation (14.9%), hyperlipidemia (2.1%), ischemic heart disease (31.9%), hemorrhagic stroke (14.9%), and ischemic stroke (31.9%) in this group of patients was obtained by reviewing the case notes and was supplemented by the autopsy findings. The causes of death were as follows: stroke (ie, ischemic stroke, hemorrhagic stroke), n=4 (8.3%); heart disease (ie, coronary artery disease, hypertensive heart disease, ), n=11 (22.9%); infection or sepsis, n=9 (18.8%); other natural causes (ie, malignancy, hepatitis, alcohol intoxication, anaphylaxis), n=16 (33.3%); unnatural causes (ie, suicides, accidents), n=8 (16.7%). Presence of brain infarctions in MCA territory was documented by previous brain computed tomography or MRI findings, or by the findings of brain cutting.

The whole brain was obtained fresh and intact during the process of autopsies. The MCAs were washed with water to clear the possible blot inside. Then the whole brain was fresh frozen at 4°C until imaged. The period between autopsy and imaging was less than 5 days.

MRI
The MRI examinations of the brains were performed by a 1.5-T scanner (Sonata, Siemens Medical Systems, Erlangen, Germany) with a standard head coil (with 8 channels). To keep the arteries uncollapsed, diluted barium solution was injected into the lumen to distend the artery. Then the brains were positioned in the scanner to resemble a supine head first position in routine clinical scanning. The orientation of the MCA was localized by the TSE T2 sequence of the whole brain. An experienced radiologist would then choose an imaging plane perpendicular to each MCA in order to obtain the cross-sectional image of individual M1 arteries. T1-weighted fat-suppressed and T2-weighted sequence was performed. The detailed parameters were referred to our previous paper.

Histopathology
The MCAs were then removed for histopathologic processing and atherosclerotic plaque classification, which was used as a reference standard to interpret the signal changes on MRI pictures. The main trunks of bilateral MCAs (ie, M1) were removed intact from the brain and sectioned into blocks. The specimen was cut into 2-cm blocks, starting from the proximal and progressing distally toward the bifurcation, until the whole length of the specimen had been cut. Serial sections of MCAs were cut at 4-mm intervals. The specimens were processed and embedded in paraffin and thereafter sections 5 m thick were cut and stained with H&E and Victoria Blue.

The histological images of all the arteries were photographed with a Leica DC 200 digital microscope (Leica Microsystems, Wetzler, Germany). The images were transferred to another computer, and the cross-sections of the vessels were traced by MetaMorph imaging system (Version 4.01) (Universal Imaging Corporative West Chester, PA).

Image Analysis
MR image quality was assessed and classified as poor, fair, good, and excellent. After excluding images of occluded vessels or poor image quality, the remaining images were quantitatively evaluated using Image-Pro Plus software (Media Cybernetics, Silver Spring, USA). The cross-sectional vessel area (VA) was measured by manually tracing the outer interface of the vessel while the luminal area (LA) was given by tracing the lumen intimal border. The plaque load (PL=VA−LA), maximum/minimum wall thickness were then derived from the VA and LA outlines. The eccentricity index was defined as (maximum wall thickness−minimum wall thickness)/maximum wall thickness. A lesion was defined as eccentric if the index was ≥0.5 and as concentric if <0.5, according to the previous study.

By using Image-Pro Plus software, the eccentricity index was calculated on the histology slides with Victoria Blue staining for every MCA, using the same methods as in MR images.

Statistical Analysis
All data analyses were conducted using the SPSS 20.0 software package (SPSS, Inc., USA). The plaque morphology of the concentric and eccentric plaque groups were compared by using independent-samples T test, and comparison of brain infarctions between groups were determined by chi-square test. The correlation of the MRI measurements with histologic findings was calculated using the Spearman correlation coefficient. A value of P<0.05 was considered to be statistically significant.

Results
Among 116 MR images (selecting the best image out of 10 images per MCA), 72 (62.1%) vessel wall images with adequate quality were selected for morphological analysis, excluding 44 images with poor quality. All these MCA lesions on MRI were verified to be atherosclerotic plaques by histology.

According to the value of calculated eccentricity index, 26 (36.1%) intracranial atherosclerotic lesions were identified as eccentric (eccentricity index ≥0.5) and 46 (63.9%) lesions as concentric on MR images. Comparatively, the eccentricity index of histology sections of 69 MCAs (3 MCAs excluded because unable to been calculated due to obvious folding during processing) was calculated. Thirty-four (49.3%) MCAs were histologically classified as eccentric plaque and 35 (50.7%) as concentric. In spite of the selection bias due to MRI imaging quality and processing artifacts caused by post-mortem shrinkage or tear of vessel specimens, the values of eccentricity index of MCA atherosclerotic lesions on MRI were correlated with corresponding values calculated from histological findings (Spearman ρ=0.637, P<0.001). Figure showed an example of concentric plaque and eccentric plaque respectively.

Table showed the comparisons of morphological features between concentric and eccentric plaques. The eccentric

![Figure. Representative magnetic resonance images and corresponding histopathologic sections of concentric and eccentric lesions. Brain magnetic resonance imaging T1-weighted fatsuppressed sequence shows examples of middle cerebral artery with concentric (A) and eccentric (B) plaques, which are verified by histopathology (C and D), respectively.](http://stroke.ahajournals.org/Downloaded-from)
plaque morphology

<table>
<thead>
<tr>
<th></th>
<th>Concentric Plaques</th>
<th>Eccentric Plaques</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel area</td>
<td>15.58±4.43</td>
<td>14.59±4.98</td>
<td>0.387</td>
</tr>
<tr>
<td>Lumen area</td>
<td>3.80±1.61</td>
<td>3.39±1.39</td>
<td>0.276</td>
</tr>
<tr>
<td>Plaque burden</td>
<td>11.77±3.48</td>
<td>11.20±3.84</td>
<td>0.519</td>
</tr>
<tr>
<td>Plaque burden/Vessel Area</td>
<td>0.756±0.08</td>
<td>0.77±0.05</td>
<td>0.395</td>
</tr>
<tr>
<td>Max wall thickness</td>
<td>1.26±0.33</td>
<td>1.93±0.44</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Min wall thickness</td>
<td>0.88±0.22</td>
<td>0.71±0.18</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Table. Plaque Morphology and Cerebrovascular Events by Eccentricity

Discussion

Our findings demonstrated that eccentric plaques account for more than half of ICAD and the eccentricity of intracranial atherosclerotic plaques may not be associated with the occurrence of brain infarctions.

Although intracranial arteries had been understudied due to the inaccessibility and invasiveness during assessment, recent advances in HRMRI techniques make it feasible to visualize intracranial arteries pathology by vessel wall imaging. Lacking of pathological evidence, however, is a big hurdle to make a definite diagnosis of variant etiologies accounting for similar presentations of intracranial vasculopathies. Therefore, it is still challenging to identify ICAD from other etiologies of intracranial vasculopathies, such as vasculitis, Moyamoya disease, dissection or reversible cerebral vasoconstriction syndrome. Currently, the feature of eccentric and irregular wall thickening is used as the criteria to identify intracranial atherosclerosis from other etiologies, whereas vasculitis is thought to be smooth and circumferential concentric wall thickening. However, our present MRI study on histology-verified ICAD found that a half or more (63.9%) of atherosclerotic lesions were concentric rather than eccentric, which suggests that pure eccentricity index is not enough to differentiate ICAD from other intracranial arterial etiologies.

An in vivo HRMRI study reported that 90% of intracranial atherosclerotic lesions showed eccentric wall thickening, much higher than that in our study. How to interpret the inconsistence between our present study and in vivo HRMRI studies? Compared to in vivo studies, the advantage of this study lies in the availability of histological evidence to determine the etiology of ischemic stroke and to make a definite diagnosis of intracranial atherosclerosis. The aim of our current study was to get the actual geometry of histology-validated intracranial atherosclerosis. There were some possibilities to explain the existing inconsistence: (1) for the in vivo studies, the uncertain diagnosis of intracranial atherosclerosis could lead to false recruitment of stroke patients with vasculitis or Moyamoya disease into ICAD-related HRMRI studies; (2) for our current study, the recruitment of general Chinese adults (>45 years old) rather than stroke patients could lead to relatively mild severity of MCA atherosclerotic lesions compared to other HRMRI studies. We deduce that the geometry of intracranial atherosclerosis may vary during the different stage of atherosclerotic progression. Accumulating data about histology-validated intracranial vasculopathies are required to clarify the morphology of ICAD. Recent clinical studies demonstrated that by using contrast-enhanced HRMRI, supplementary information about the enhancement patterns may provide extra valuable evidence for definite diagnosis of ICAD. Multicontrast vessel wall imaging can be a complementary tool for intracranial vasculopathy differentiation, which often leads to more invasive workups for definite diagnosis.

As for clinical relevance of plaque eccentricity, a clinical study on carotid plaques suggested that eccentric plaque is a potential marker for ipsilateral cerebrovascular events. Our present study on histology-verified ICAD, however, failed to indicate the association between eccentric intracranial atherosclerotic plaques and brain infarctions, in spite of seemingly higher incidence of brain infarction due to eccentric plaques rather than concentric plaques. Further studies are needed to clarify the morphological features of ICAD and to explore their clinical relevance.

This study has some limitations. Firstly, the relative low resolution of 1.5T MRI may affect the imaging quality, which could not provide enough information of plaque components. Our previous study validated the feasibility of ex-vivo 1.5T MRI in identifying MCA stenosis and in detecting intraplaque hemorrhage of intracranial atherosclerotic lesions in one special case. The application of 3T or 7T HRMRI would provide better images to visualize the vessel wall pathology of ICAD. Secondly, the selection of the best MRI-section of the MCA (one section only) to calculate plaque geometry based on imaging quality could affect the eccentricity index calculation. To overcome the limitation, the global eccentricity index was also calculated based on histology analysis. Thirdly, as stated above, the different inclusion criteria between our current histology-based study and in vivo HRMRI may partly account for the inconsistent patterns of eccentricity. Moreover, the recruitment of autopsy cases with other potential origins of brain infarctions (ie, cardioembolism, carotid atherosclerotic disease) rather than symptomatic intracranial disease could affect the patterns.

To our knowledge, this is the first study to evaluate plaque eccentricity of histology-verified ICAD using 1.5T MR imaging. Further imaging studies with improved resolution will be needed.
required to investigate the morphological features of ICAD for differentiating variant disease etiologies.

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**Disclosures**
None

**References**
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