ABSTRACT

Objectives
Identify determinants associated with variability in collateral status in patients with acute ischemic stroke and test if collateral status on CTA can be used to select patients for intra-arterial therapy (IAT).

Methods
Data are from the Keimyung Stroke Registry. Patients with M1 MCA occlusion on baseline CTA from 5/2004 to 7/2009 were included. All imaging was analyzed blinded to clinical information.

Results
Multivariable modeling identified metabolic syndrome (OR 3.22 95% CI 1.69-6.15, p<0.001), hyperuricemia (per 1 mg/dl OR 1.35 95% CI 1.12-1.62, p<0.01) and older age (per 10 years, OR 1.34 95% CI 1.02-1.77, p=0.03) as independent predictors of poor collateral status.

Only patients with intermediate or good collaterals who recanalize show association with good clinical outcome. (Rate Ratio=3.8, 95% CI 1.2-12.1).

Conclusions
Metabolic syndrome, hyperuricemia and age are associated with poor collateral status. Patients with poor collaterals on CTA do not benefit from IAT.
ACKNOWLEDGEMENTS

This work is a testament to a beautiful friendship I share with Sung Il Sohn; a friendship that developed not through the medium of spoken language, but through empathy, compassion and a common love for knowledge.

This work is also testament to the relationship I share with my mentor and now friend, Michael (Hill); a relationship born out of admiration for a person whose passion for stroke, for epidemiology and bio-statistics and above all for doing the right thing has inspired me all these years.

My interest in stroke imaging is primarily due to one person: Andrew (Demchuk). Andrew is the best teacher I ever had. Mayank (Goyal) has been my mentor and now my best friend. The umpteen hours spent with Mayank arguing, debating and working on stroke imaging have been some of the best times of my life. To Eric (Smith) and Richard (Frayne) from whom I learnt diligence, hard work, and attention to detail, I express my sincerest gratitude. To Drs. Wiebe and Kaplan, I express thanks for being such wonderful role models. To Vivek (Nambari), I express my thanks for those long hours working with me. Vivek was first author and I was senior author on the work described in Chapter 5.

This work would not have been possible without the patients whose suffering helps inform our research. I hope and pray that this work and everything I do helps my patients.

To Archana and Hari; you give my life and everything I do purpose. To my parents; Thanks for dreaming. I would have achieved nothing without those dreams. To my brother; You are my Hero.
To My Family

*In the vastness of space and the eternity of time, it gives me great joy to share an epoch and a planet with you.*

*Modified from a quote by Carl Sagan*
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACA</td>
<td>anterior cerebral artery</td>
</tr>
<tr>
<td>ASPECTS</td>
<td>Alberta Stroke Program Early CT Score.</td>
</tr>
<tr>
<td>CTA</td>
<td>CT angiography.</td>
</tr>
<tr>
<td>ED</td>
<td>Emergency department.</td>
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<tr>
<td>IAT</td>
<td>intra-arterial therapy.</td>
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<tr>
<td>ICA</td>
<td>intracranial internal carotid artery.</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range.</td>
</tr>
<tr>
<td>IV tPA</td>
<td>intravenous tissue plasminogen activator.</td>
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<tr>
<td>MCA</td>
<td>middle cerebral artery.</td>
</tr>
<tr>
<td>mRS</td>
<td>modified Rankin Scale.</td>
</tr>
<tr>
<td>NCCT</td>
<td>non-contrast CT.</td>
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<tr>
<td>NIHSS</td>
<td>National Institute of Health stroke scale.</td>
</tr>
<tr>
<td>PCA</td>
<td>posterior cerebral artery</td>
</tr>
<tr>
<td>PCT</td>
<td>Perfusion CT</td>
</tr>
<tr>
<td>rLMC</td>
<td>regional leptomeningeal collateral score.</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation.</td>
</tr>
<tr>
<td>TICI</td>
<td>Thrombolysis in Cerebral Infarction score.</td>
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</table>
1.1 Introduction

**Leptomeningeal collaterals** are native (pre-existing) anastomoses that cross-connect a small number of distal-most arterioles within the crowns of the cerebral artery trees. These collaterals represent a potentially under-recognized, emergency “backdoor” to maintain blood flow to the brain that would otherwise die during an acute ischemic stroke. Marked variation in retrograde perfusion amongst patients with acute ischemic stroke.
suggests wide variability in the presence of these collaterals in humans.\textsuperscript{1,3,4} Very few studies have sought to understand the basis for this variability, although in animal studies a wide variation in density and diameter of these vessels has been shown to be associated with environmental factors (e.g. presence of cardiovascular risk factors) and genetic background.\textsuperscript{5-8} Knowledge of the sources of this variation in humans may permit identification of therapeutic targets capable of modulating native collateral status, thereby achieving better clinical outcomes in patients with ischemic stroke.

Current imaging techniques are incapable of directly visualizing these collaterals in humans. Assessment of leptomeningeal collateral status therefore relies on measuring blood flow distal to a blocked cerebral artery.\textsuperscript{4,9} These techniques include CT-angiography, perfusion CT and conventional cerebral angiograms. CT-angiography scales are the most widely used because of ease of availability and acquisition, lower radiation and relative non-invasiveness.\textsuperscript{1,2,4,10-12} Construct validity of CT-angiography based scales have been demonstrated by correlation with cerebral blood flow and prognostic value in determining clinical outcomes in patients with acute ischemic stroke.\textsuperscript{2,9,13}

In this proposal, I seek to identify potentially modifiable and non-modifiable determinants of variability in leptomeningeal collateral status in patients with acute ischemic stroke and proximal occlusions. In addition, I seek to understand the role of leptomeningeal collaterals in determining clinical outcome in this population and whether imaging of collaterals can be used to select patients for intra-arterial revascularization therapy. Leptomeningeal collateral status in these patients will be measured using single phase CT-angiography of the head.
1.2 **Primary Hypothesis**

Pre-existing modifiable cardiovascular risk factors (namely hypertension, diabetes mellitus, metabolic syndrome and hyperuricemia) are associated with poor leptomeningeal collateral status in patients presenting with acute ischemic stroke.

1.3 **Secondary Hypothesis**

Patients with good and intermediate collaters on single-phase CTA head achieve good clinical outcome with early recanalization; patients with poor collaters do not do well even with early recanalization.

1.4 **Objectives**

- Identify modifiable and non-modifiable determinants of poor collateral status in patients with acute ischemic stroke.
- Determine if patients with acute ischemic stroke will have a differential clinical response to early recanalization based on collateral status assessed on single phase CTA.

1.5 **Thesis Overview**

A background review regarding leptomeningeal collaterals, their anatomy and physiology, reasons for variability in their status, imaging techniques to measure them and inherent drawbacks in each such technique are covered in Chapter 2. A description of the database used in this proposal as well as a brief description of the data elements and methods of data storage is provided in Chapter 3. Chapter 4 describes in detail methods employed to test
the primary hypothesis (thus identifying determinants of poor collateral status in patients with acute ischemic stroke), the results of this analysis and discussion of these results. This work is published in the Annals of Neurology (Leptomeningeal collaterals are associated with modifiable metabolic risk factors. Ann Neurol 2013;74:241-248). Chapter 5 describes analyses that seek to demonstrate if collaterals modify the association between recanalization after endovascular therapy. Methods, results and a detailed discussion on the clinical relevance of the results follow. This work is published in the American Journal of Neuroradiology (CTA collateral status and response to recanalization in patients with acute ischemic stroke. AJNR 2014;35:884-890). Concluding notes and suggestions for future research are discussed in Chapter 6.
CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction

There exists substantial variability in severity of stroke symptoms at presentation. This variability at presentation is explained by differences in etiology, eloquence of affected brain, site of arterial occlusion and sensitivity of neurons to hypoxia. However, a large part of the variability in stroke severity occurs when patients with occlusions at the same site present with strokes of varying severity. Leptomeningeal collaterals are the sole source of perfusion to ischemic brain distal to an occlusion. Variability in stroke severity could potentially be explained by variability in the presence of these collaterals. Using single-phase CTA Head examination, our group and others have previously shown that approximately 15-40% of patients with acute ischemic stroke have poor native collaterals and more than 1/3rd tend to have abundant collaterals at baseline. We have also shown evidence for variability in the presence of inter-territorial (between MCA-ACA and MCA-PCA) and intra-territorial (within MCA) collaterals in humans.

2.2 Measuring variability in leptomeningeal collaterals in humans

While studies in animal models directly visualize leptomeningeal collaterals, direct visualization of these vessels in humans using current imaging modalities is not possible. Since collaterals mediate retrograde tissue perfusion in the presence of an arterial occlusion, we and others have developed techniques to qualitatively measure the extent or rate of retrograde backfilling of pial arteries. Measuring extent and/or rate of retrograde
filling pial arteries is therefore a reliable surrogate measure of leptomeningeal collateral status.\textsuperscript{4,10,11}

Conventional cerebral angiography is considered the reference standard for assessment of leptomeningeal collaterals using this principle. It is however an invasive test; not performed at presentation in many patients with acute ischemic stroke.\textsuperscript{26,27} Even if undertaken, comprehensive assessment of collateral status using catheter angiography would require a detailed four vessel study; a procedure often neither available, feasible or appropriate in an acute stroke scenario where time is of essence. Perfusion imaging has been used to define collateral status in a few studies; this technique however requires sophisticated post processing and time to interpret images.\textsuperscript{11,28} Patient motion can also result in errors in measurement.\textsuperscript{28} CT Angiography (CTA) is capable of measuring retrograde filling of pial arteries distal to an occlusion in patients with acute ischemic stroke. It is quicker and simpler to obtain in an acute stroke setting than other imaging modalities. It is also relatively resistant to patient motion. Collateral assessment on single phase CTA Head examination has, in addition, shown good inter-reliability.\textsuperscript{4} In this proposal, we will use a single phase CTA Head based collateral assessment scale that we have previously developed and validated.\textsuperscript{4} The scale is described below and in Figure 2-1 and Figure 2-2. The scale is compared to other available scales on CTA/DSA in Table 2-1.

2.3 The regional Leptomeningeal Collateral Score (rLMC score) on CTA:

The rLMC score\textsuperscript{4} (20 points) is based on scoring extent of contrast opacification in arteries distal to an M1 MCA+- ICA occlusion (0, artery not seen; 1, less prominent; 2, equal or
more prominent compared with a matching region in the opposite hemisphere) in 6
cortical regions (M1 to M6), parasagittal ACA territory, and the basal ganglia (*Figure 2-1*
and *Figure 2-2*). Lenticulostriate arteries in the basal ganglia arising from retrograde filling
MCAs distal to an occlusion are included in the scoring. Arteries in the Sylvian sulcus are
given a higher score, i.e. 0, 2, or 4 (0, not seen; 2, less; 4, same or prominent compared with
the opposite Sylvian sulcus) because opacification of these vessels most distant from
leptomeningeal anterior cerebral to MCA and posterior cerebral to MCA anastomoses is a
strong indicator of good retrograde flow via these collateral networks. Higher total scores
indicate better collateral status. Since the rLMC score is based on an anatomical template,
inter-rater reliability is excellent (Intra-class correlation coefficient, 0.87; 95% CI, 0.77%–
0.95%). The rLMC score can only measure collateral status in patients with M1 MCA +/-
distal ICA occlusions.
Figure 2-1: Regional Leptomeningeal Collateral Score on CT angiography – “Excellent Collaterals”.

A 65-year-old female presenting with dysarthria, left hemiparesis and ipsilateral facial paresis (NIHSS = 12). Imaging shows a right proximal M1 MCA occlusion with excellent leptomeningeal collaterals including retrograde opacification of the pial arteries until the distal end of the thrombus. Regional Leptomeningeal Score (rLMC score) is 19.
Figure 2-2: Regional Leptomeningeal Collateral Score on CT angiography – “Poor Collaterals”.

A 70-year-old male with expressive aphasia, left hemiplegia with left hemi-sensory loss (NIHSS = 22). Imaging shows evidence of carotid “T occlusion” with cross filling of the ipsilateral A2 via an anterior communication artery. Poor leptomeningeal collateral status results in poor visualization of vessels in the left frontal and parietal regions. Note backfilling of pial arteries in the Sylvian sulcus. Regional Leptomeningeal Score (rLMC score) is 8.
Table 2-1: Imaging sequence and methods used in scoring leptomeningeal collaterals using CTA or conventional angiography.

<table>
<thead>
<tr>
<th>Schramm, 2002</th>
<th>Imaging Sequence</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTASI 2mm slice</td>
<td>Degree of perilesional vessel enhancement, primarily in sylvian sulcus</td>
<td></td>
</tr>
<tr>
<td>Kim, 2004</td>
<td>DSA</td>
<td>Collateral vessels in 15 sites as per a modified ASPECTS template</td>
</tr>
<tr>
<td>Christoforidis, 2006</td>
<td>DSA</td>
<td>Extent of retrograde backfilling of pial arteries on AP view</td>
</tr>
<tr>
<td>Tan, 2007</td>
<td>CTA MIPS, 20mm</td>
<td>Degree of collateral vessel enhancement (enhancing vessels within the total vascular territory supplied by the occluded arterial segment)</td>
</tr>
<tr>
<td>Rosenthal, 2008</td>
<td>CTASI, 3mm</td>
<td>Degree of leptomeningeal and sylvian vessel enhancement</td>
</tr>
<tr>
<td>Maas, 2009</td>
<td>CTASI</td>
<td>Degree of leptomeningeal and sylvian vessel enhancement</td>
</tr>
<tr>
<td>Menon, 2011</td>
<td>CTA MIPS 40mm</td>
<td>Size and extent of retrograde backfilling pial arteries on a regional (modified ASPECTS) template.</td>
</tr>
</tbody>
</table>

2.4 Factors that determine variability in leptomeningeal collateral status at baseline in patients with acute ischemic stroke:

Environmental factors have been shown to impact leptomeningeal collateral extent. Ageing and endothelial nitric oxide synthase (eNOS) deficiency [and potentially other classic cardiovascular risk factors] cause reduction in collateral density and diameter (collateral “rarefaction”), resulting in larger infarct volume after ischemic stroke. Animal studies have shown that naturally occurring differences at distinct genomic loci cause large differences in the density and diameter of leptomeningeal collaterals (and smaller differences in length and tortuosity). Remodeling of collaterals to arteries with larger
lumen and better functional capacity could potentially affect native collateral status at baseline when triggered by a previous stroke or transient ischemic attack (TIA). Collaterogenesis triggered by the presenting stroke itself and variations in this may take days to manifest, if indeed it occurs and cannot explain variation in native collateral status in patients presenting with acute ischemic stroke.\textsuperscript{32} Below, I seek to describe many potential modifiable and non-modifiable determinants. (Table 2-2) This discussion informs the primary hypothesis that I seek to test in this proposal.

Table 2-2: Variables potentially associated with collateral status in patients with acute ischemic stroke.

<table>
<thead>
<tr>
<th></th>
<th>Non modifiable</th>
<th>Modifiable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Blood Glucose at baseline</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>Glycosylated Hb</td>
</tr>
<tr>
<td></td>
<td>Diabetic status</td>
<td>Systolic and diastolic blood pressure at baseline</td>
</tr>
<tr>
<td></td>
<td>Hypertensive status</td>
<td>Lipid Panel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum Homocysteine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum Uric acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use of statins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use of Angiotensin Converting Enzyme inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use of Nitrates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use of antiplatelets</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obesity and the metabolic syndrome</td>
</tr>
<tr>
<td></td>
<td>Markers of vascular inflammation</td>
<td>High sensitivity-C-Reactive Protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum fibrinogen</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>d-Dimer levels</td>
</tr>
</tbody>
</table>
2.4.1 Modifiable determinants

Collateral extent can be impaired in the coronary and peripheral vascular bed in the presence of cardiovascular risk factors (hypertension, diabetes, dyslipidemia, smoking).\textsuperscript{33} Cardiovascular risk factor-associated impaired collateral extent could involve any of the multiple steps: depressed mobilization of bone marrow-derived cells, decreased elaboration of angiogenic factors from these cells or diminished responsiveness of remodeling collaterals to angiogenic stimuli. Many cardiovascular risk factors are associated with impaired endothelial Nitric Oxide Synthase (eNOS) and endothelial function. Endothelial dysfunction has also been associated with accelerated collateral rarefaction and more severe stroke in animal studies.\textsuperscript{36}

Microvascular rarefaction has long been known to occur with hypertension.\textsuperscript{34} Studies using animal models (spontaneously hypertensive rats) have found evidence of decrease in pial arteriolar external diameter, encroachment of the internal arteriolar lumen and rarefaction\textsuperscript{35-37} potentially mediated either by the renin angiotensin system or by activation of the sympathetic nervous system\textsuperscript{38,39} Reversibility of this type of remodeling with Angiotensin Converting Enzyme (ACE) inhibitors but not with vasodilators like hydralazine support this mechanism.\textsuperscript{39} Preliminary studies in mice have found that chronic genetic renin-dependent hypertension causes rarefaction of collaterals and increased stroke severity.\textsuperscript{35} Some studies have shown an association between poor collateral status and history of hypertension in humans.\textsuperscript{19} Other studies have shown an association between higher systolic blood pressure at baseline and poor leptomeningeal collateral status.\textsuperscript{19,40} The latter association could be a result of physiological stress during stroke; nonetheless a
causal association with hypertension cannot be ruled out. Hypotension at presentation may lead to reduction in functional capacity of collaterals and poor clinical outcomes.\textsuperscript{15}

**Hyperglycemia** is common in patients with acute stroke, typically occurring in 20-25\% of stroke patients overall (but up to 60\% in some populations), and in approximately 12-53\% of acute stroke patients without a prior diagnosis of diabetes.\textsuperscript{41} It has been associated with increased stroke severity and mortality. Although hyperglycemia has often been attributed to the physiological stresses following stroke, it could reflect underlying impaired glucose tolerance or diabetes mellitus. Abnormal glycosylated Hemoglobin (HbA1c) is observed in 42\% of patients presenting with stroke and without a known prior diagnosis of diabetes mellitus.\textsuperscript{42}

Acute hyperglycemia might limit collateral status by actively promoting the constriction of leptomeningeal arteries.\textsuperscript{43,44} Such a constriction could arise from the ability of elevated glucose to inhibit voltage gated K\(^+\) channels in vascular smooth muscle\textsuperscript{43,45} or impair the production of endothelial derived relaxing factors like nitric oxide.\textsuperscript{44,46} Acute hyperglycemia appears to influence these downstream targets by inducing the production of reactive oxygen species or by promoting a secondary inflammatory process.\textsuperscript{44,45} Microvascular rarefaction occurs in diabetes mellitus\textsuperscript{34,47}, thus such an effect could also result in reduction in native pial collateral extent. Preliminary animal studies support this hypothesis. Human studies have however not been able to show a relationship between presence of diabetes mellitus and poor collateral status.\textsuperscript{19,48} A limitation has been lack of information on control of blood sugar prior to stroke using such measures as glycosylated Hb. A comprehensive analysis of this association would entail having data on pre-existing
type and duration of diabetes mellitus, glycosylated Hb and serum glucose levels at baseline and 24 hours.

Metabolic syndrome is a cluster of risk factors - elevated blood pressure, dyslipidemia, and central obesity, associated with various biological manifestations, including hyperglycemia and insulin resistance. Patients with metabolic syndrome have an increased risk for cardiovascular morbidity and mortality. These patients also have endothelial dysfunction, decreased circulating adiponectin and heightened expression of plasminogen activator inhibitor-1 (PAI-1) that may negatively influence vascular growth. Circulating adiponectin and PAI-1 influence collateral remodeling and angiogenesis by stimulating endothelial progenitor cell migration and inhibiting apoptosis. Hypercholesterolemia and insulin resistance are also associated with poor collateral remodeling in the coronary vasculature. Preliminary studies in animal models of metabolic syndrome have found rarefaction in cerebral and hind-limb collaterals. However sub-components of the metabolic syndrome like fasting plasma glucose or blood pressure can be affected by stroke severity itself and not be causal.

There is a growing body of evidence linking raised serum uric acid to cardiovascular risk and increased arteriolar stiffness. Endothelial dysfunction and vascular smooth muscle proliferation are hypothesized as possible mechanisms. Interestingly, recent evidence suggests that hyperuricemia is an independent predictor of increased arteriolar stiffness in normal Korean men.

The use of statins pre-morbidly has been associated with good pre-existing leptomeningeal collaterals in patients with acute ischemic stroke. This association between statin use and
good native leptomeningeal collateral status could possibly be due to the effect of statins in enhancing nitric oxide mediated vasodilatation by activating endothelial nitric oxide synthase (eNOS).\textsuperscript{40} \textit{Glyceryl trinitrate and other similar organic nitrates} are also known to cause NO mediated cerebral vasodilatation and either maintain or potentially augment estimated cerebral perfusion pressure even when lowering mean arterial pressure.\textsuperscript{58-60} 

\textit{Serum homocysteine, serum fibrinogen, vascular endothelial growth factor (VEGF)}\textsuperscript{61}, \textit{angiotensin (AT1) receptor blockade}\textsuperscript{62}, \textit{catecholamines}\textsuperscript{63} and \textit{pCO\textsubscript{2}} may impact collateral status in the coronary and peripheral vascular beds.\textsuperscript{64} Association of other variables mentioned above with collateral status has not been studied in humans. 

\textit{Ischemic pre-conditioning} may favor collaterogenesis based on studies in coronary vascular beds.\textsuperscript{65} Evidence for this phenomenon is also seen in brain of animal models; nonetheless evidence in human brain is at best weak.\textsuperscript{66} Potential mechanisms include cellular defense function against ischemia enhanced by posttranslational modification of proteins or by expression of new proteins that strengthen the influence of survival factors or inhibit apoptosis, synthesis of stress proteins that may lead to an increased capacity for cell survival or increased collaterogenesis.\textsuperscript{66} A possible association between pre-existing carotid disease, previous TIAS/minor strokes, leukoaraiosis and good leptomeningeal collateral status is hypothesized.\textsuperscript{67,68}
2.4.2 Non-modifiable determinants

*Aging* is associated with poor clinical outcome in patients with acute ischemic stroke.\(^{69}\) Animal studies have found that increasing age is associated with collateral rarefaction, increased resistance of the collateral network, impaired remodeling and larger stroke, changes that are accompanied by endothelial nitric oxide synthase (eNOS) deficiency.\(^{6}\) Targeted disruption of eNOS greatly accelerates age-associated collateral rarefaction.\(^{29}\) Studies in patients with acute ischemic stroke have shown conflicting results, with some reporting weak association of poor leptomeningeal collateral status with ageing and others showing no relationship.\(^{4,19,40}\)

There is increasing evidence of *sex*-specific differences in stroke incidence, severity and disability.\(^{70}\) Animal models in the peripheral vasculature have shown decreased angiogenesis and collaterogenesis in female after unilateral hind limb ischemia with no difference in pre-existing vessel number at baseline.\(^{71}\) However, no effect of sex was found in the Candq1 genetic locus in mice, although parent-of-origin effects were found (maternal parent’s genotype at the locus was associated with collateral extent in the offspring). Nonetheless, evidence on sex disparities in native collateral status in humans is missing.

2.4.3 Genetic polymorphism

Recent studies in mice indicate that inter-individual variability in leptomeningeal collateral status are due to genetic- and/or environmental-dependent differences in native collateral number and diameter or capacity to outwardly model.\(^{5,7}\) A 3-fold difference in number and 30-fold difference in diameter of leptomeningeal collaterals has been noted between inbred
mice with different genetic backgrounds. These differences closely correlate with similar large differences in infarct volume after MCA occlusion. This wide variation in collateral extent in mice has been linked to a quantitative trait locus on chromosome 7 (Canq1, LOD 30). A different locus on chromosome 11 (Carq1) is linked to variation in collateral modeling. Gene targeting studies have identified 5 genes, Vegf-a, Clic-4, Flk1, Adam10 and Adam17 that are associated with differences in collateral formation in the embryo and thus collateral extent in the adult. Gene polymorphisms and differences in expression of interferon-β, galectin-2, and PNPLA4, MAP2K4, DCP1A and ACTN1 have been associated with differences in coronary collateral status in humans. Importantly, variation in extent of leptomeningeal collaterals in mice is shared by similar variation in collateral extent in other tissues. Since this may also be true in humans, the above human and mouse genes provide rationale targets for study in patients with acute ischemic stroke. No current studies on genetic variability in leptomeningeal collateral status in humans have been done.

In summary therefore, there is biological evidence for an association between the various modifiable and non-modifiable variables discussed above and poor native leptomeningeal collaterals. In this thesis, for the first time, using a large acute ischemic stroke database in humans, these variables will be assessed systematically for association with poor leptomeningeal collateral status. By identifying modifiable determinants of collaterals, this thesis will help focus future research on potential interventions aimed at improving native collateral status preventively before stroke onset or in the hyper-acute phase after stroke onset.
2.5 Imaging of leptomeningeal collateral status at baseline and association with clinical outcome:

Acute treatment of ischemic stroke is aimed at *salvaging viable but ischemic brain* by recanalizing the occluded artery and restoring anterograde perfusion as *quickly* as possible. Time saved while making critical decisions correctly is vital in acute ischemic stroke management. Imaging techniques that can identify presence of salvageable brain rapidly and efficiently are therefore an important first step in acute stroke management.

Several imaging paradigms propose to “select” patients with acute ischemic stroke suitable for recanalization therapy. Sophisticated *MRI techniques* have been touted as the solution but remain unproven and have practical drawbacks. MRI takes up to 30 minutes to screen, perform and interpret. Many patients do not tolerate it as well as CT; image quality is affected by patient motion. MRI also has limited availability “after hours”.

*CT perfusion* is used by many centers for patient selection. Limitations of CT perfusion include time (it takes 10 to 30 minutes from image acquisition to interpretation) post-processing algorithms that are vendor specific, not standardized and therefore variable across centers, the need for trained personnel to process these images. In addition, image quality may be affected by patient motion, and radiation dose poses a small safety hazard in some patients.

We and others have shown that good collateral status at baseline measured on single phase CTA Head examination results in reduced infarct volume and good clinical outcome on follow-up. Good collateral status at baseline also results in higher reperfusion...
rates with thrombolytic therapy and higher chance of achieving good clinical outcome with endovascular therapy.\textsuperscript{4,89,90} The odds of achieving recanalization with good collateral status was 4.6 times that with poor collateral status;\textsuperscript{89} the odds of achieving good clinical outcome (mRS 0-2 at 90 days) with endovascular therapy was 4.8 times that with poor collateral status.\textsuperscript{4} Patients with poor collateral status at baseline have poor imaging and clinical outcomes.\textsuperscript{91} Patients with favorable leptomeningeal collaterals show better outcomes because there is greater access to thrombus for thrombolytic agents, reduced ischemia severity and maintenance of vessel wall integrity through retrograde perfusion.\textsuperscript{1,13}

In summary, leptomeningeal collaterals can only be measured indirectly in humans. Although techniques like MRI and CT Perfusion are surrogate (indirect) markers of leptomeningeal collateral status in patients with acute ischemic stroke, there are practical limitations to performing these imaging techniques in the acute stroke milieu. Single phase CTA Head examination is a simple, reliable technique to measure leptomeningeal collaterals in these patients. Collateral assessment using all these techniques correlates with clinical outcomes and is therefore an excellent prognostic tool in these patients.

2.5.1 \textit{Collateral imaging as an imaging selection tool to select patients for endovascular therapy:}

Of note therefore, as discussed in the previous section, evidence exists on the prognostic role of collateral status (as measured by many imaging techniques) in determining clinical outcome in patients with acute ischemic stroke. There is however \textit{minimal evidence on whether collateral imaging can be used to select patients for endovascular therapy.} To
demonstrate the utility of collateral imaging as a selection tool to help select the right patients for endovascular therapy, demonstration that patients with good collaterals benefit more when the therapy is offered (and is successful) than when it is not while patients with poor collaterals do not benefit even if therapy is offered successfully is needed. Only then can collateral imaging be used to select patients for this therapy. In summary, demonstration of effect modification is required i.e. collateral status on imaging modifies the relationship between successful therapy (early recanalization) and good clinical outcome.

The implications are many because the tool – single phase CTA head examination defined collateral vessel assessment - itself is cheap, readily available and quick. A large number of patients could benefit from appropriate patient selection. It is also possible that collateral assessment on single phase CTA head may complement perfusion CT by identifying patients with worse hypoperfusion within penumbral thresholds identified by perfusion CT, thereby identifying how rapidly penumbral tissue progresses to infarction. An additional advantage of CTA over perfusion CT will be the ability to identify thrombus length/burden more precisely.

The recently published consensus statement on acute stroke imaging by the Stroke Imaging Research Group (STIR) recommends that an ideal imaging selection tool be reliable, widely available and not delay treatment initiation. Imaging of collaterals using single phase CTA head examination is one such tool.
CHAPTER 3: THE KEIMYUNG STROKE REGISTRY, DATABASE AND DATA ELEMENTS

3.1 Introduction

Data are from the Keimyung Stroke Registry, an ongoing single center retrospective/prospective observational study of patients with acute ischemic stroke presenting to the Keimyung University Hospital in Daegu, South Korea. Keimyung University is one of the leading academic universities in South Korea. All imaging analysis was done at the Seaman MR Center and the Calgary Stroke Program. All imaging was read and scored by two readers (BKM and SIS) by consensus. A major advantage of the Keimyung Stroke Registry when compared to other similar stroke registries is its size and the fact that it has prospectively collected data on many potential determinants of leptomeningeal collaterals discussed above. For testing our hypothesis, we included only patients presenting with acute ischemic stroke with M1 segment middle cerebral artery (MCA) +/- intracranial internal carotid artery (ICA) occlusions on baseline CT-angio. We have all imaging and clinical data for the time period May 2004 to July 2009 for the proposed study. The local ethics board of Keimyung University has approved the study.

3.2 Clinical Data

All patients included in the study were imaged with a non-contrast CT head at admission followed by CT-angiography of the head and neck. Information on demographic and clinical characteristics, medical history, admission physical examination findings including height, weight and waist circumference, and laboratory parameters including complete blood count, blood glucose levels, INR, C-reactive protein (CRP), and erythrocyte sedimentation...
rate (ESR), were collected at baseline. Fasting blood sugar, glycosylated hemoglobin (Hb), serum uric acid (on fasting sample), homocysteine, fibrinogen, D-dimer and lipid panel were collected the next morning. Stroke severity was assessed using the National Institute of Health Stroke Scale (NIHSS) at baseline, immediately after treatment, at discharge and at 90 days. Functional status was assessed using the modified Rankin Scale (mRS) at similar time-points. Interval times from stroke symptom onset to presentation in emergency room (ER), imaging, thrombolysis and endovascular procedures are also collected. All biochemical measurements are in SI units. All data are de-identified and contained in a password-protected database.

3.3 Imaging Methodology

At Keimyung University Hospital, Standard non-helical non-contrast CT (NCCT) was performed on a multi-slice scanner (Siemens, Forchheim, Germany) using 120 kV, 170 mAs with 5-mm slice thickness. NCCT was followed by CT Angiography with a helical scan technique. Coverage was from arch to vertex with continuous axial slices parallel to the orbitomeatal line with 0.6 mm to 1.25 mm slice thickness. Acquisitions were obtained after a single bolus intravenous contrast injection of 90-120 ml nonionic contrast media into an antecubital vein at 3-5 ml/sec, auto-triggered by appearance of contrast in a region of interest manually placed in the ascending aorta. The data were then anonymized (all relevant DICOM headers removed) before being stored onto a password protected hard-drive. SIS then uploaded the data onto a dedicated workstation and database at the SeamanMR center.
The Seaman MR center has a dedicated imaging database unit and provided secure image collection and core imaging laboratory services for our study.

Baseline and follow-up imaging were analyzed at the imaging core lab of the Seaman MR center and the Calgary Stroke Program. OsiriX version 3.5 (http://www.osirix-viewer.com) was used to reconstruct 2D multi-planar reconstruction images in axial, coronal, and sagittal planes using 24-mm-thick slabs.

**Leptomeningeal collaterals were assessed on baseline CT-angio** by consensus (BKM, SIS) using the regional leptomeningeal score (rLMC). (See Chapter 2; Figure 2-1 and Figure 2-2). Both readers were blinded to all clinical information and follow-up data at the time of reading the scans. All patients had follow-up imaging using NCCT/MRI at 24 hours and at time of discharge. Recanalization (opening of occluded artery) after endovascular therapy was determined on conventional cerebral angiogram at end of endovascular procedure on repeat MR Angiogram at 24 hours.

**Recanalization** was defined as TICI 2b or 3 on conventional angiogram or as disappearance of occluding thrombus with clearly visible convexity pial arteries on MRA. The TICI (Thrombolysis In Cerebral Infarction) scale was proposed in 2003 as a modification of the TIMI scale used in the coronary vascular bed. TICI categories include no perfusion (grade 0), “partial perfusion” category (grade 2) sub-divided into grade 2a (<50% of the ischemic territory is reperfused) and 2b (>50% of the ischemic territory is reperfused) and grade 3 (normal reperfusion of ischemic territory).
3.4 Final clinical outcome:

Final clinical outcome was determined using the modified Rankin Scale (mRS) at 3 months. (Appendix 1) The mRS is a 7-point scale that measures neurological functional disability and is the de facto standard outcome measure for stroke clinical trials.\textsuperscript{93} We therefore used functional ability as assessed by this scale at 90 days as our primary clinical outcome. Good clinical outcome was defined as a mRS score of 0-2 at 90 days post stroke. In the Keimyung Registry, nurses or resident physicians who were blinded to patient treatment at baseline did mRS assessment.

3.5 Broad Inclusion and Exclusion Criteria

Specific inclusion and exclusion criteria applicable to each specific study are mentioned in the appropriate chapters.

3.5.1 Inclusion Criteria for proposed Study:

1) Any patient presenting to the emergency department with symptoms consistent with ischemic stroke within 12 hours of stroke symptom onset.
2) Evidence of a visible and symptomatic intracranial occlusion on baseline CT-angiography (M1 MCA segment +/- intracranial ICA).

3.5.2 Exclusion Criteria

1) Intracranial hemorrhage (ICH) identified on baseline CT.
2) Previous moderate to large stroke in the ipsilesional hemisphere.
3) Modified Rankin Scale > 2 at baseline.
4) Participation in another study that results in the patient receiving an investigational drug or therapy.
CHAPTER 4: LEPTOMENINGEAL COLLATERALS ARE ASSOCIATED WITH MODIFIABLE METABOLIC RISK FACTORS

4.1 Background

Leptomeningeal collaterals are pre-existing anastomoses that cross-connect a small number of distal-most arterioles within the crowns of the cerebral artery trees. These collaterals represent potential endogenous bypass vessels capable of maintaining blood flow to brain regions that would otherwise die during an acute ischemic stroke. There is robust evidence from imaging studies among patients with acute ischemic stroke to show that the extent of these collaterals at baseline exhibits substantial variability and that patients with fewer collateral vessels have worse outcomes. The determinants of variability in collateral abundance in patients with acute ischemic stroke are largely unknown. However, animal studies point to genetic polymorphisms in genes that control formation of the collateral circulation in tissues during development, as well as environmental factors (e.g. ageing, chronic endothelial dysfunction) that are associated with thinning of these collaterals and more severe tissue injury in models of ischemic stroke and peripheral artery disease.

Previous pilot studies have shown weak associations between a history of hypertension and poor collaterals, and between use of statins and good collaterals. Age, ischemic preconditioning and presence of cardiovascular risk factors are all hypothesized as

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1 This chapter is published as a manuscript in the Annals of Neurology 2013;74:241-248.
determinants of native collateral status.\textsuperscript{4,6,29} We sought to examine potential determinants of native leptomeningeal collaterals in large cohort of well-studied acute ischemic stroke.

4.2 Methods

Data are from the Keimyung Stroke Registry, an ongoing single center observational study of patients with acute ischemic stroke presenting to the Keimyung University Hospital in Daegu, South Korea. We included patients presenting with acute ischemic stroke with M1 segment middle cerebral artery (MCA) +/- intracranial internal carotid artery (ICA) occlusions on baseline CT-angio during the time period May 2004 to July 2009 in the study. All patients undergo a non-contrast CT head at admission followed by CT-angiography of the head and neck. Information on demographic and clinical characteristics, medical history, admission physical examination findings including height, weight and waist circumference, and laboratory parameters including complete blood count, blood glucose levels, INR, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), are collected at baseline. Fasting blood sugar, glycosylated hemoglobin (Hb), serum uric acid (on fasting sample), homocysteine, fibrinogen, D-dimer and lipid panel are collected the next morning. Stroke severity is assessed using the National Institute of Health Stroke Scale (NIHSS) at baseline, immediately after treatment, at discharge and at 90 days. Functional status is assessed using the modified Rankin Scale (mRS) at similar time-points. Interval times from stroke symptom onset to presentation in emergency room (ER), imaging, thrombolysis and endovascular procedures are also collected. The local Institutional Review Board approved the study.
In 71 patients who either woke up with or did not have witnessed onset of stroke symptoms, we imputed stroke onset time as midpoint between “last seen normal time” and “time discovered with stroke symptoms”. Metabolic syndrome was defined as the presence of 3 or more of the following components: (1) serum triglycerides $\geq 150$ mg/dL; (2) HDL cholesterol $< 40$ mg/dL for men and $< 50$ mg/dL for women; (3) fasting plasma glucose $> 110$ mg/dL or use of anti-diabetic medication; (4) blood pressure $\geq 130/85$ mm Hg or medication use and (5) abdominal obesity. Abdominal obesity was defined as per the revised Asia-Pacific criteria suggested by the World Health Organization Asia Pacific Region.

Standard non-helical NCCT was performed on a multi-slice scanner (Siemens, Forchheim, Germany) using 120 kV, 170 mAs with 5-mm slice thickness. NCCT was followed by CTA with a helical scan technique. Coverage was from arch to vertex with continuous axial slices parallel to the orbitomeatal line with 0.6 mm to 1.25 mm slice thickness. Acquisitions were obtained after a single bolus intravenous contrast injection of 90-120 ml nonionic contrast media into an antecubital vein at 3-5 ml/sec, auto-triggered by appearance of contrast in a region of interest manually placed in the ascending aorta. Baseline and follow-up imaging was analyzed at the imaging core lab of the Seaman MR center and the Calgary Stroke Program. OsiriX version 3.5 (http://www.osirix-viewer.com), an image processing software designed for multi-planar reconstruction and volume rendering was used to reconstruct 2D multi-planar reconstruction images in axial, coronal, and sagittal planes using 24-mm-thick slabs. Leptomeningeal collaterals were assessed on baseline CT-angiography by consensus (BKM, SIS) using the regional leptomeningeal score (rLMC). This ordinal 20
point score assesses pial arterial enhancement within the total vascular territory supplied
by the occluded arterial segment to similar maximal enhancement on the opposite
unaffected hemisphere using an ASPECTS based template. The score has demonstrated
good inter-rater reliability. Both readers were blinded to all clinical information and
follow-up data at the time of reading the scans.

**Statistical Analyses:** Data are reported using conventional descriptive statistics. Potential
predictor variables were determined based on causal relevance and availability of data
(Table 4-1). Less than 5% of data for candidate predictor variables were missing except for
glycosylated hemoglobin (Hb); therefore glycosylated Hb was excluded from multivariable
analyses. Otherwise, missing data were imputed to the median value except for height,
weight and waist circumference where missing data were imputed to the sex-specific
median value. The primary outcome was the rLMC score dichotomized as 0-10 (poor) vs.
11-20 (good). Sensitivity analyses without imputed data were also performed and did not
show any difference in results (data not shown). Predictor variables showing significance
(p<0.05) in the above analyses were assessed for clinical relevance. Blood glucose and
raised white blood cell count that are potentially causal but also hypothesized to increase
with increased stress (due to stroke severity and poor collateral status at baseline) were
excluded from further analyses. In addition, multi-collinearity was assessed using
Spearman’s ranked correlation and Bonferroni’s correction for multiple comparisons. Only
variables identified as statistically significant in univariate analyses (p<0.05) and
hypothesized as causal were included in multivariable logistic regression modeling.
Variables showing correlation (r ≥ 0.25) and therefore possibly not independent of each
other were not included in the same model. Two-way multiplicative interactions were explored between relevant predictor variables included in the model. Model building was done manually using a combination of backwards elimination and forward selection until a parsimonious model was achieved which includes only predictor variables conventionally significant at $\alpha=0.05$. We tested for an interaction between metabolic syndrome and time from stroke symptom onset to CT time (dichotomized as $\leq 90$ minutes and $> 90$ minutes) in predicting poor collateral status, reasoning that if accumulating stroke-related stress increases blood pressure and serum glucose causing false “metabolic syndrome”, then an association between apparent “metabolic syndrome” and poor collaterals might be seen in the later time window. Since leptomeningeal collateral status is measured on CT-angiography using an ordered categorical scale (rLMC score), similar models as above were retested using a proportional odds approach where the outcome is analyzed as per 4 different thresholds for poor collateral status (cut 1: rLMC score 0-15 vs. 16-20, cut 2: rLMC score 0-10 vs. 11-20 and cut 3: rLMC score 0-5 vs. 6-20). The proportional odds assumption tested using the Brant test was satisfied. ($p=0.2$). All $p$-values are two-sided, with $p<0.05$ considered statistically significant. Analyses were performed using Stata/SE 12.1 software (Copyright 1985-2011 StataCorp LP).

4.3 Results

During the study period, there were 286 patients with documented anterior circulation occlusions on baseline CT-angiography. After excluding 43 patients with a documented MCA occlusion beyond the M1 MCA segment, 28 patients with isolated ICA without M1
MCA occlusion, 7 patients with simultaneously detected occlusions in the posterior circulation or contralateral ICA territory and 2 patients with poor image quality, 206 patients were included for analyses. The baseline characteristics and outcomes are described in Table 4-1. Thirty-eight patients (18.5%) were treated conservatively, 79 (38.4%) received IV t-PA with or without endovascular therapy and 89 (43.2%) were treated with endovascular therapy alone. Significant correlation was noted between baseline ASPECTS on NCCT and collateral status (Spearman's correlation coefficient r=0.54, p<0.0001).
Table 4-1: Variables stratified by good vs. poor baseline leptomeningeal collateral status on CT-angio (n=206). * Lab parameters measured at presentation.

<table>
<thead>
<tr>
<th>Leptomeningeal Collaterals</th>
<th>Good (rLMC score 11-20) (n=133)</th>
<th>Poor (rLMC score 0-10) (n=73)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (in yrs, mean +/- SD)</td>
<td>65.6 +/- 12.2</td>
<td>69.1 +/- 10.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex (male, %)</td>
<td>54.90%</td>
<td>47.90%</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>Baseline Clinical Measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height in cm (IQR)</td>
<td>163 (14)</td>
<td>162.5 (13)</td>
<td>0.84</td>
</tr>
<tr>
<td>Weight in Kg (IQR)</td>
<td>60 (11)</td>
<td>60 (13)</td>
<td>0.34</td>
</tr>
<tr>
<td>Waist in cm (IQR)</td>
<td>82 (12)</td>
<td>81 (23)</td>
<td>0.96</td>
</tr>
<tr>
<td>Body Mass Index in kg/m2 (IQR)</td>
<td>22.5 +/- 4</td>
<td>22.9 +/- 3.02</td>
<td>0.38</td>
</tr>
<tr>
<td>Systolic Blood Pressure +/- SD (mmHg)</td>
<td>136.5 +/- 27.2</td>
<td>143.1 +/- 29.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Diastolic Blood Pressure +/- SD (mm Hg)</td>
<td>82.7 +/- 12.8</td>
<td>83.6 +/- 13.5</td>
<td>0.63</td>
</tr>
<tr>
<td>Temperature (IQR) (celsius)</td>
<td>36.4 (0.6)</td>
<td>36.2 (0.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Onset to CT time (median, IQR) (mins)</td>
<td>137.5 (147.5)</td>
<td>136.0 (119)</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Pre-morbid Risk Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>47.4%</td>
<td>64.4%</td>
<td>0.02</td>
</tr>
<tr>
<td>Coronary arterial disease (%)</td>
<td>34.2%</td>
<td>24.8%</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>27%</td>
<td>37.0%</td>
<td>0.15</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>34.1%</td>
<td>31.5%</td>
<td>0.41</td>
</tr>
<tr>
<td>Previous stroke/TIA (%)</td>
<td>18.6%</td>
<td>23.3%</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Laboratory Studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Glucose +/-SD (mg/dL)*</td>
<td>133.1+/-.48.1</td>
<td>164.5 +/- 95.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hemoglobin in g/dL* (IQR)</td>
<td>13.2 (2.1)</td>
<td>13.7 (2.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>Hematocrit (%)*</td>
<td>38.4 (7.3)</td>
<td>40.3 (6.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>WBC count in cells/mL* (IQR)</td>
<td>7780 (3280)</td>
<td>8460 (3940)</td>
<td>0.02</td>
</tr>
<tr>
<td>Platelet count in cells per mL* (IQR)</td>
<td>235000 (93000)</td>
<td>230000 (72000)</td>
<td>0.27</td>
</tr>
<tr>
<td>ESR mm/hr* (IQR)</td>
<td>11 (14)</td>
<td>12 (17)</td>
<td>0.98</td>
</tr>
<tr>
<td>CRP in mg/L* (IQR)</td>
<td>0.38 (1.01)</td>
<td>0.38 (1.17)</td>
<td>0.85</td>
</tr>
<tr>
<td>INR IU* (IQR)</td>
<td>1 (0.12)</td>
<td>1 (0.12)</td>
<td>0.29</td>
</tr>
<tr>
<td>D-dimer mcg/mL (IQR)</td>
<td>1 (2.1)</td>
<td>2.25 (4.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum Fibrinogen mg/dL (IQR)</td>
<td>253 (103)</td>
<td>268 (99)</td>
<td>0.18</td>
</tr>
<tr>
<td>Serum Homocysteine μmol/L (IQR)</td>
<td>10.52 (5.7)</td>
<td>11.02 (5.7)</td>
<td>0.46</td>
</tr>
<tr>
<td>Serum Uric acid mg/dL (IQR)</td>
<td>4.8 (2.5)</td>
<td>5.3 (2.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total Cholesterol mg/dL (IQR)</td>
<td>175 (64)</td>
<td>180 (42)</td>
<td>0.38</td>
</tr>
<tr>
<td>Triglycerides mg/dL (IQR)</td>
<td>83.8 (59.1)</td>
<td>90.6 (47.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>HDL-C mg/dL (IQR)</td>
<td>45.3 (14.5)</td>
<td>46.1 (13.9)</td>
<td>0.73</td>
</tr>
<tr>
<td>LDL-C mg/dL (IQR)</td>
<td>103.5 (52.3)</td>
<td>117.3 (29.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>21.50%</td>
<td>46.60%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRS 0-2 at 90 days</td>
<td>40.60%</td>
<td>19.20%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Patients with poor collateral status at baseline were older, more frequently hypertensive and had higher blood glucose values and higher white blood cell (WBC) count at baseline. They also had higher D-dimer and serum uric acid levels (measured the following morning) and were more likely to be labeled as having metabolic syndrome. (Table 4-1). We explored the relationship of A) metabolic syndrome, serum uric acid and hypertensive status and B) metabolic syndrome, serum uric acid and D-dimer levels to poor collateral status. D-dimer levels are known to be associated with history of hypertension; so these variables were not included in the same model.100 Both models A and B included three predictor variables, two-way interactions between these predictor variables and a term testing for joint modification of “metabolic syndrome” by the other two variables. Multivariable modeling using both model A and B identified metabolic syndrome and raised serum uric acid as independent predictors of poor leptomeningeal collateral status at baseline. Hypertensive status and serum D-dimer levels did not modify or confound the association between metabolic syndrome, serum uric acid and the outcome variable (poor collateral status). The final model using forward selection included age in addition to metabolic syndrome and serum uric acid and identified all three as significant independent predictors of poor collateral status (Table 4-2). Age did not modify or confound the association between metabolic syndrome and raised serum uric acid with poor collateral status. A sensitivity analyses restricted to patients with isolated M1 MCA occlusions showed similar results (Table 4-3). We also did not find a statistically significant interaction between metabolic syndrome and stroke onset to CT time in a model including serum uric acid and age as the other predictor variables. (Likelihood ratio statistic, p=0.99).
Table 4-2: Final model predicting poor leptomeningeal collateral status (rLMC score 0-10) using logistic regression and forward selection. (n=206)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>Lower 95% of OR</th>
<th>Upper 95% of OR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic Syndrome</td>
<td>3.22</td>
<td>1.69</td>
<td>6.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum Uric Acid (per 1 mg/dL)</td>
<td>1.35</td>
<td>1.12</td>
<td>1.62</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (per 10 years)</td>
<td>1.34</td>
<td>1.02</td>
<td>1.77</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Table 4-3: Final model (Sensitivity Analyses) predicting poor leptomeningeal collateral status (rLMC score 0-10) using logistic regression and forward selection in the subgroup of patients with isolated M1 MCA occlusions. (n=116)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>Lower 95% of OR</th>
<th>Upper 95% of OR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic Syndrome</td>
<td>2.17</td>
<td>1.24</td>
<td>5.21</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum Uric Acid (per 1 mg/dL)</td>
<td>1.43</td>
<td>1.08</td>
<td>1.88</td>
<td>0.01</td>
</tr>
<tr>
<td>Age (per 10 years)</td>
<td>1.48</td>
<td>1.02</td>
<td>2.23</td>
<td>0.04</td>
</tr>
</tbody>
</table>

In a sensitivity analysis using a proportional odds approach, we tested the final model and found similar results across 3 different cuts for the outcome (poor collateral status defined as cut 1: rLMC score 0-15 vs. 16-20, cut 2: rLMC score 0-10 vs. 11-20 and cut 3: rLMC score 0-5 vs. 6-20) (Table 4-4). The parallel regression assumption for this model was not violated as per Brant’s test (p=0.2) suggesting that metabolic syndrome, raised uric acid and age were independent predictors of poor collateral status (defined in 3 different ways on an ordered categorical scale).
Table 4-4: Proportional odds model predicting poor leptomeningeal collateral status
(cut 1: rLMC score 0-15 vs. 16-20, cut 2: rLMC score 0-10 vs. 11-20 and cut 3: rLMC score 0-
5 vs. 6-20). Brant test p value = 0.16 suggesting that the parallel regression assumption has
not been violated for this model. (n=206)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>Lower 95% of OR</th>
<th>Upper 95% of OR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic Syndrome</td>
<td>2.27</td>
<td>1.25</td>
<td>4.11</td>
<td>0.007</td>
</tr>
<tr>
<td>Serum Uric Acid (per 1 mg/dL)</td>
<td>1.25</td>
<td>1.08</td>
<td>1.46</td>
<td>0.003</td>
</tr>
<tr>
<td>Age (per 10 years)</td>
<td>1.51</td>
<td>1.19</td>
<td>1.92</td>
<td>0.002</td>
</tr>
</tbody>
</table>

β₀ coefficients with 95% CI for poor collaterals at each pre-specified cut
/cut 1  | 2.6 | 0.84 | 4.37 | N/A |
/cut 2  | 4.89 | 3.01 | 6.76 | N/A |
/cut 3  | 6.65 | 4.68 | 8.61 | N/A |
4.4 Discussion

In this study, we identified the presence of metabolic syndrome, hyperuricemia and ageing as independently associated with poor baseline leptomeningeal collateral status among patients presenting with acute ischemic stroke. Variability in native leptomeningeal collateral status can to a large extent be explained by genetic and environmental determinants. Animal studies have been particularly informative, demonstrating the presence of genetic determinants of variation in collateral extent and that natural ageing and experimentally induced endothelial dysfunction cause a reduction in the density of native collaterals in brain and other tissues.\textsuperscript{5-8,29} Although studies in the coronary vascular bed in humans have suggested that cardiovascular risk factors including dyslipidemia, hyperglycemia, hypertension, smoking, obesity and ageing may contribute to variability in collateral extent, many of the associations remain uncertain, and causal mechanisms unknown.\textsuperscript{101,102} Studies examining the determinants of variability in native leptomeningeal collateral status in humans have been limited.\textsuperscript{4,40,94} History of hypertension and higher systolic blood pressure on admission have been associated with poor collateral status at baseline, and statin use with good collateral status, in patients presenting with acute ischemic stroke, although the evidence has not been compelling.\textsuperscript{19,40}

Patients with metabolic syndrome are proposed to have insulin resistance, endothelial dysfunction, decreased circulating adiponectin and heightened expression of plasminogen activator inhibitor-1 (PAI-1) that may negatively influence vascular remodeling and potentially cause microvascular rarefaction and reduction in cerebral arteriolar diameter (external and luminal).\textsuperscript{103,104} Likewise, only a preliminary report addresses this question in
experimental studies, finding in a mouse genetic model of chronic metabolic syndrome that the native collateral circulation in brain and hind-limb experiences rarefaction (decline in collateral density and diameter), resulting in more severe tissue loss in models of ischemia. Ours is the first study to report on an association between metabolic syndrome and poor leptomeningeal collateral status in humans.

There is a growing body of evidence linking raised serum uric acid to cardiovascular risk and increased arteriolar stiffness. Endothelial dysfunction and vascular smooth muscle proliferation are hypothesized as possible mechanisms. Hyperuricemia is known to be associated with hypertension, chronic kidney disease, insulin resistance and the metabolic syndrome. Controversy still exists as to whether it is a marker of these diseases or a causal factor. Our results suggest that hyperuricemia is associated with poor collateral status after adjusting for age, presence of hypertension, serum D-dimer level and metabolic syndrome. Recent evidence that hyperuricemia is an independent predictor of increased arteriolar stiffness in normal Korean men potentially supports the view that it is causal and reduces collateral status by either causing endothelial dysfunction or decreasing dilatatory capacity of the pial arteries.

We are also able to confirm findings from animal studies that increasing age results in worsening native collateral status. Experimental studies have led to the suggestion that endothelial Nitric Oxide Synthase (eNOS) derived nitric oxide is a maintenance factor for collateral vessels, resulting in their rarefaction with prolonged endothelial dysfunction.
Acute hyperglycemia adversely affects stroke outcome.\textsuperscript{106} It could potentially have a primary role in determining leptomeningeal collateral status. We did find an association between hyperglycemia and poor collateral status on univariate analyses. We did not however have data on glycosylated Hb in more than 10% of our patients. Glycosylated Hb is an indicator of raised blood sugar before stroke symptom onset and is less likely to be affected by stroke severity than blood glucose measured after stroke onset. The current study was unable to distinguish between raised blood glucose prior to stroke symptom onset that could affect collateral status and raised blood sugar after stroke onset possibly as a result of increased stress due to the stroke itself. We did not, however, find any association between previous diabetic status and poor collaterals.

Chronic hypertension was not a significant predictor of poor collaterals after adjusting for presence of metabolic syndrome and hyperuricemia. Patients with metabolic syndrome may already have the pathological milieu for hypertension even when there is no clinically detected hypertension. Animal literature suggests that chronic hypertension impairs dilatation of collateral blood vessels in the cerebral circulation.\textsuperscript{107} In a recent study, spontaneously hypertensive rats had poor collaterals when compared to normotensive rats; but had increased collateral growth and improved blood flow to ischemic brain after treatment with Angiotensin II AT 1 receptor blockade.\textsuperscript{39} Patients with metabolic syndrome or chronic hypertension could potentially improve collateral status with such treatment.

Elevated D-dimer levels are hypothesized to potentiate pro-inflammatory cytokines and possibly cause endothelial dysfunction.\textsuperscript{108} We did not find any evidence for its association
with poor collateral status. We also did not find any role of sex in determining leptomeningeal collateral status.\textsuperscript{71}

Our study has limitations. We did not have access to pre-stroke fasting plasma glucose and blood pressure recordings, and therefore used values collected on presentation to the emergency room, instead. These subcomponents of the metabolic syndrome could be affected by the stroke itself.\textsuperscript{109} However, we did not find an increasing prevalence of metabolic syndrome with increasing time from stroke symptom onset, which would be expected if accumulating stroke related stress results in raised plasma glucose and blood pressure. There is, in addition, evidence to suggest that lipid levels remain stable in the first few days of an acute ischemic stroke and are not associated with an acute phase reactant response.\textsuperscript{110,111} Given that metabolic syndrome is only diagnosed if 3 or more of 5 components are present, we think that substantial over-estimation of the metabolic syndrome is unlikely. We cannot entirely exclude the possibility that hyperuricemia could simply be a marker of more severe strokes or, potentially, a neuro-protective response to stroke.\textsuperscript{105} However, our findings are consistent with a growing body of evidence that suggests that hyperuricemia could inhibit collateral function by causing endothelial dysfunction.\textsuperscript{54,55,57} Our patient cohort is East Asian; as such our findings may only apply to the population we studied. Our study needs to be validated in other patient cohorts. Nonetheless, our study is the largest to date examining determinants of leptomeningeal collateral status in detail.

Our study findings are hypothesis generating and warrant confirmation in independent studies. Metabolic syndrome and hyperuricemia are modifiable determinants of collateral
status. Exercise, high fiber and low fat diet, weight loss, metformin, thiazolidinediones, statins, angiotensin receptor blockade, low purine diet, xanthine oxidase inhibitors and uricosuric agents are all potential interventions whose role in improving native collateral status could be studied in future. The knowledge thus gained could focus research on clinical trials and appropriate therapeutic strategies to enhance leptomeningeal collaterals or augment their function either preventively before stroke onset, or in the acute phase after stroke onset.
CHAPTER 5: CTA COLLATERAL STATUS AND RESPONSE TO RECANALIZATION IN PATIENTS WITH ACUTE ISCHEMIC STROKE

5.1 Background

Leptomeningeal collaterals are pre-existing anastomoses that connect a small number of distal-most arterioles within the crowns of the cerebral artery trees. During an acute stroke, ischemic brain depends on blood flow from these collaterals to survive until the occluded artery is opened. This collateral circulation is highly variable and potentially influences the rate at which an infarct grows. Collateral status at baseline is an independent determinant of clinical outcome among patients with acute ischemic stroke. Nonetheless, “effect modification” by collateral status measured non-invasively using CT-angio (CTA) of the relationship between recanalization and clinical outcome has not been demonstrated before. It is only by demonstrating a differential clinical response to recanalization by collateral status methodically that this tool can be used to select patients for intra-arterial therapy (IAT).

In this study, we first demonstrate concept validity of collateral status measured using CTA among patients presenting with acute ischemic stroke by correlating it with infarct volume on baseline MR DWI and infarct growth over 24 hours. We then demonstrate “effect modification” by collateral status of the relationship between recanalization and clinical outcome.

This chapter is published as a manuscript in the American Journal of Neuroradiology AJNR 2014;35:884-890.
outcome in patients with acute ischemic stroke undergoing intra-arterial therapy, thus justifying the use of baseline collateral status on CTA as a patient selection tool for intra-arterial therapy.

5.2 Methods

The Keimyung Stroke Registry is an ongoing single center observational study of patients with acute ischemic stroke presenting to the Keimyung University Hospital in Daegu, South Korea. All patients undergo a non-contrast CT (NCCT) head at admission followed by CTA of the head and neck. For the study (time period May 2004 to July 2009), we included only those patients presenting with acute ischemic stroke with M1 segment middle cerebral artery (MCA) +/- intracranial internal carotid artery (ICA) occlusion on baseline CTA with witnessed stroke symptom onset, who were treated with IAT and had a pre-treatment MRI brain and a 24 hour post-treatment MRI.

During this time period, there were 286 patients with documented anterior circulation occlusions on baseline CTA. We excluded 202 patients (43 patients with a documented MCA occlusion beyond the M1 MCA segment, 28 patients with isolated ICA without M1 MCA occlusion, 7 patients with simultaneously detected occlusions in the posterior circulation or contralateral ICA territory, 71 patients with unknown stroke onset time, 16 patients with baseline MRI obtained post treatment, 29 patients with poor quality MRI at baseline and 8 patients without 24 hour MRI). Finally, 84 patients were included in these analyses. Stroke severity was assessed using the National Institute of Health Stroke Scale (NIHSS) at baseline, immediately after treatment, at discharge and at 90 days. Interval
times from stroke symptom onset to presentation in Emergency room (ER), imaging and endovascular procedures were collected. Functional status was assessed at baseline and 90 days using the modified Rankin Scale (mRS). We collected data on mRS by clinical review in a face-to-face interview at 3 months (in about 70% of patients) and by telephone interview at 3 months (in about 25% of patients). For 5% patients, we obtained the 3-month mRS information at 5-9 months. The local Institutional Review Board approved the study.

**Imaging protocol:** Standard non-helical NCCT was performed on a multi-slice scanner (Siemens, Forchheim, Germany) using 120 kV, 170 mAs with 5-mm slice thickness. NCCT was followed by CTA with a helical scan technique. Coverage was from arch to vertex with continuous axial slices parallel to the orbitomeatal line with 0.6 mm to 1.25 mm slice thickness. Acquisitions were obtained after a single bolus intravenous contrast injection of 90-120 ml nonionic contrast media into an antecubital vein at 3-5 ml/sec, auto-triggered by appearance of contrast in a region of interest manually placed in the ascending aorta. This was followed by a baseline MRI (3 Tesla, Signa Exite, General Electronic, Milwaukee, WI) consisting of DWI, time of flight MRA and GRE as a part of stroke protocol. Follow-up MRI was done within 24 hours using the same protocol.

**Intra-arterial therapy (IAT):** IV tPA was given to all eligible patients within 3 hours of stroke onset as per accepted guidelines. MRI was primarily used to assess intracerebral hemorrhage on GRE and recanalization status on MR TOF. Patients were taken to the angiography suite from MRI suite for IAT. Patients with MCA occlusions with or without ICA occlusions who did not show recanalization on MRA or rapid clinical improvement after IV tPA were considered for IAT. IAT included local urokinase (10,000–20,000 IU/min;
maximum permissible dose, 300,000 IU; Green Cross Pharm., Seoul, Republic of Korea), balloon angioplasty or wire manipulation. Glycoprotein IIb/IIIa receptor antagonists were given in a small number of patients. For clots not manageable by the above methods, we used off-label stenting (using coronary stents). Recanalization was assessed by angiography in all cases. Successful recanalization was defined as TICI 2b/3 flow on final angiogram. Penumbra Stroke System aspiration catheters and stentriever devices (eg. Solitaire FR™, Trevo™) were not available during this period.

**Imaging Analyses:** Baseline and follow-up images were analyzed at the imaging core lab of the Calgary Stroke Program. OsiriX version 3.5 (http://www.osirix-viewer.com), an image processing software designed for multi-planar reconstruction was used to reconstruct 2D multi-planar reconstruction images of CTA in axial, coronal, and sagittal planes using 24-mm-thick slabs. Leptomeningeal collaterals were assessed on baseline CTA by consensus (BKM, SIS) using the regional leptomeningeal score (rLMC); a previously published ordinal scoring system based on the ASPECTS template that has excellent inter-rater reliability.4

(Figure 5-1) Infarct volumes were measured on baseline and 24 hour DWI MR using an in-house validated software, Quantomo (Cybertrial Inc. Calgary, CA). Infarct growth over 24 hours was calculated by subtracting initial infarct volume from the 24-hour volume measurement. Quantomo has been previously validated and has good inter-rater and intra-rater agreement.113 Readers were blinded to all clinical information and follow-up data at the time of reading the scans.
Figure 5-1. Collateral status on CTA as measured by the rLMC score with baseline and 24 hour infarct volume on MR DWI.

**Statistical Analyses**: Continuous variables are summarized as means (+/- 1 SD) or medians (IQR or range) as appropriate. Collateral status using the rLMC score (0-20) was trichotomized into 3 groups: good (17-20), intermediate (11-16) and poor (0-10) as per previously published literature. Differences between these 3 groups were assessed using Fisher’s exact test for proportions, one-way ANOVA for parametric data and a rank sum test for non-parametric data. We tested for trend in outcome measure (infarct growth and mRS 0-2 at 90 days) by collateral status in the recanalizers (TICI 2b-3) and in the non-
recanalizers (TICI 0-1) using Cuzick’s test of trends. We then built a multivariable model using generalized linear modeling with a log link with mRS 0-2 vs. 3-6 at 90 days as the outcome and collateral status (good, intermediate and poor), recanalization status (TICI 2b-3 vs. 0-2a) and age (by decile) as independent variables. Since there was no significant association of sex, baseline NIHSS, onset to imaging time with mRS at 90 days; these variables were not included in the model. We specifically included a multiplicative interaction term between collateral status and recanalization status. All hypothesis tests are two-sided, with p<0.05 considered statistically significant. In multivariable analysis, interaction effects were considered significant at p<0.10. Analyses were performed using Stata/SE 12.1 software (Copyright 1985-2011 StataCorp LP).

5.3 Results

Among 84 patients, mean age was 65.2±13.2 years, median NIHSS was 14 (IQR 8.5) and median time from stroke onset to initial MRI was 164 minutes. Successful recanalization (TICI 2b-3) was achieved in 38.1% patients and good clinical outcome (mRS 0-2 at 90 days) in 35.8% patients. Mean baseline infarct volume was 58.5 ml (+/- 1 SD 64.4 ml) and mean infarct growth over 24 hours was 58.3 ml (+/- 1 SD 72 ml). There were 19 patients with good, 34 with intermediate and 31 with poor collateral scores at baseline. Baseline characteristics stratified by good, intermediate and poor collateral status are described in Table 5-1. Patients with good collateral status had lower baseline NIHSS, higher baseline ASPECTS on NCCT, smaller baseline infarct volume on MRI and lesser infarct growth over 24 hours. (Table 5-1)
Infarct growth over 24 hours was significantly lower in those patients with good collateral status at baseline who achieved recanalization (7.0 ml; +/- 1 SD=11.7 ml) when compared to those with intermediate collateral status who achieved recanalization (26.6 ml; +/- 1 SD=43.4 ml) and those with poor collaterals who recanalized (67.7 ml; +/- 1 SD=75.1 ml) (p=0.05; between group difference). There was no difference in infarct growth in the non-recanalizers stratified by collateral status (good collaterals: 58.4 ml, +/- 1 SD=58.6 ml, intermediate collaterals: 48.6 ml, +/- 1 SD=63.7 ml, poor collaterals: 100.4 ml, +/- 1 SD=91 ml, p=0.09; between group difference). (Figure 5-2).
Figure 5-2: Baseline infarct volume and infarct growth over 24 hours stratified by baseline collateral status on CTA and final recanalization (TICI 2b-3 vs. 0-1).

Similarly, good clinical outcome (mRS 0-2 at 90 days) was higher among patients with good collateral status who achieved recanalization (100%) when compared to those with intermediate collateral status who recanalized (58.8%) and those with poor collaterals who recanalized (33.3%) (p=0.04). There was no statistically significant difference in good clinical outcome in the non-recanalizers stratified by collateral status (30.8% in those good collaterals, 17.6% in those with intermediate collaterals and 18.2% in those with poor collaterals; p=0.67). Cuzick's test of trend by collateral status was statistically significant
for good clinical outcome (mRS 0-2 at 90 days, p=0.01) in the recanalizers. There was no statistically significant trend by collateral status for good clinical outcome (p=0.08) in the non-recanalizers. (*Figure 5-3*)

**Figure 5-3:** 90-day clinical outcome as per the modified Rankin Scale stratified by baseline collateral status on CTA and final recanalization (TICI 2b-3 vs. 0-1).

In the multivariable model, the interaction between collateral status and recanalization was relevant (p=0.08). This final model also included age as a significant independent predictor of good clinical outcome. Given presence of a statistically significant interaction
in the model, we report age and sex adjusted rate ratios for patients in 4 groups namely group 1 (poor collaterals who do not recanalize, n=22), group 2 (poor collaterals who recanalize, n=9), group 3 (intermediate or good collaterals who do not recanalize, n=30), and group 4 (intermediate or good collaterals who recanalize, n=23). Patients with intermediate and good collaterals were collapsed into one group for ease of reporting interaction effects. Only patients with intermediate or good collaterals who recanalize show a statistically significant association with good clinical outcome. (Rate Ratio=3.8, 95% CI 1.2-12.1). None of the other groups do as well clinically. (Table 5-2) Figure 5-3 shows the unadjusted mRS scores at 90 days in these 4 groups stratified by recanalization status.

**Table 5-2: Generalized linear model using log link showing rate ratios for good clinical outcome (mRS 0-2 at 90 days) by collateral status and recanalization status adjusted for age and sex. (Reference group consists of patients with poor collaterals who did not recanalize).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Rate Ratio</th>
<th>95% CI</th>
<th>(mRS 0-2 at 90 days, %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor Collaterals, Non-Recanalizers (n=22)</td>
<td>1</td>
<td>_</td>
<td>18.2%</td>
<td>_</td>
</tr>
<tr>
<td>Poor Collaterals, Recanalizers (n=9)</td>
<td>2</td>
<td>0.5-8.3</td>
<td>33.3%</td>
<td>0.34</td>
</tr>
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<td>Intermediate and Good Collaterals, Non-Recanalizers (n=30)</td>
<td>1.6</td>
<td>0.5-5.5</td>
<td>23.3%</td>
<td>0.44</td>
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<td>Intermediate and Good Collaterals, Recanalizers (n=23)</td>
<td>3.8</td>
<td>1.2-12.1</td>
<td>70%</td>
<td>0.02</td>
</tr>
<tr>
<td>Age (for every 10 years below 80)</td>
<td>1.2</td>
<td>1.1-1.4</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>0.6</td>
<td>0.4-1.1</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

mRS = modified Rankin Scale, CI = Confidence Interval
5.4 Discussion

Our results show that collateral status measured at baseline using CTA among patients with acute ischemic stroke correlates with baseline infarct volume and with infarct growth over 24 hours. Further, we demonstrate effect modification by collateral status of the relationship between recanalization and good clinical outcome. Patients with good and intermediate collaterals who achieve recanalization with IAT do well when compared to those who do not achieve recanalization. Patients with poor collaterals do not do well even if recanalization achieved with IAT. (Table 5-2)

Several imaging paradigms propose to “select” patients most suitable for IAT. MRI based diffusion/perfusion mismatch is one such paradigm. MRI, however, takes time to screen, perform and interpret. Many patients do not tolerate it as well as CT; image quality is affected by patient motion. MRI also has limited availability “after hours”. Moreover, the recent MR Rescue trial showed that the tool may not help in selecting patients for IAT. NCCT ASPECTS has moderate inter-rater reliability even amongst experts. Reliability is less in the early presenters (within 90 minutes from stroke symptom onset). In addition, ASPECTS interpretation is affected by patient motion and in the aged. CT perfusion needs algorithms for post processing images that are vendor specific, not standardized and therefore variable across centers. Trained personnel are sometimes needed to process these images. In addition, image quality is affected to some extent by patient motion. Additional radiation dose is also a concern. Collateral assessment on CTA has good inter-rater reliability. Good correlation with baseline infarct volume and infarct growth on MR DWI in our study demonstrates the tool’s content
validity. Further, by demonstrating “effect modification” by collateral status among patients undergoing IAT, we demonstrate for the first time that this tool can be used to select patients for this therapy. Collateral assessment on CTA does not need any sophisticated algorithm or trained personnel for post processing images and is relatively resistant to patient motion induced artifacts. It is available 24*7 hours in most centers. We therefore propose the use of this tool for patient selection within future endovascular trials.

In spite of the variability in measuring collaterals at baseline in patient with acute ischemic stroke, our study further reinforces evidence from previous literature that patients with good collaterals at baseline have small baseline infarcts when compared to patients with intermediate and poor baseline collaterals. Recanalization helps reduce further infarct growth, thus limiting size of final infarct. Our study also shows that the rate of infarct growth is quicker in patients with intermediate collaterals; these patients may only benefit if recanalization is achieved quickly. Patients with poor collaterals at baseline grow their infarcts the quickest; the likelihood that they will benefit from recanalization is the least. Nonetheless, the fact that some patients with poor collaterals in our study achieved good clinical outcome raises the possibility that routine CTA may have underestimated good and intermediate collaterals in some patients, misclassifying these collaterals as poor. Routine CTA is a single snap shot of contrast filled blood vessels. Early timing of image acquisition with respect to that of bolus injection could potentially result in under-estimation of true collateral status using this technique. The four patients who achieved good clinical outcome in spite of having poor collaterals at baseline and not achieving recanalization had a mean baseline infarct volume of 63.1 ml (range 13.3 ml to 133.5 ml) when compared to a
mean baseline infarct volume of 110 ml for all patients with poor collaterals in the study, thus suggesting possible misclassification of good and intermediate collateral status as poor using routine CTA in at least those patients with small or intermediate baseline infarct volume.\textsuperscript{123} The rLMC score used in our study being more liberal when defining poor collaterals in comparison to stricter definitions used in other studies could also explain why some patients with poor collaterals did well in our study.\textsuperscript{91} Finally, variability in brain eloquence or possible misclassification of 90-day clinical outcome due to phone follow-up in around 25\% of patients could potentially explain why two patients with poor baseline collaterals and baseline infarct volumes > 80 ml in our study did well clinically.\textsuperscript{123} Both these patients had right hemispherical infarcts. Our study, however, is able to show with a degree of statistical certainty that the patients most likely to benefit from recanalization are those with good and intermediate and baseline collaterals; patients with poor collaterals do not show a differential response to recanalization.

Reperfusion related edema and injury has been postulated to be a cause for increased infarct growth among patients without mismatch who achieve reperfusion.\textsuperscript{82} In our study, patients with poor collateral status who did not recanalize had more infarct growth than patients with poor collateral status who recanalized. Our results do not support the reperfusion injury hypothesis. On the contrary, we speculate that early recanalization, even among patients with poor collaterals, may reduce the risk of mortality.\textsuperscript{124}

Our study is not a randomized controlled trial. Similar to previous studies supporting the use of a “mismatch” based paradigm on perfusion imaging, our study provides evidence for the use of a “CTA collateral assessment based paradigm” in selecting patients for IAT.\textsuperscript{82} We
did not have data on every procedural time metric; around 30% of follow-up clinical data (mRS at 90 days) was ascertained by phone. We were also underpowered to do a secondary analysis on the effect of time to recanalization on clinical outcomes stratified by collateral status. Our recanalization rates are modest and reflective of the time period when patients were recruited; nonetheless our study is unique in that we were able to show a statistically significant interaction between collateral status and recanalization. With the advent of stentrieversthat achieve recanalization rates in excess of 80%, future demonstration of such statistically significant “effect modification” may need very large cohorts.124 Our study is thus timely in being able to demonstrate the utility of this tool in patient selection for IAT.

In conclusion, results from our study suggest a differential clinical and imaging response to recanalization by IAT in patients with good and intermediate collaterals. Patients with poor collaterals show no such differential response. Collateral assessment on CTA can potentially help aid patient selection for acute intra-arterial stroke treatment and for potential inclusion in endovascular stroke trials.
CHAPTER 6: FUTURE STUDIES

6.1 Understanding causal pathways in brain ischemia

In chapter 4, we showed that ageing, the presence of the metabolic syndrome and hyperuricemia are associated with poor collateral status in patients with acute ischemic stroke. Our analysis used *a priori* independent variables (established from animal studies), that each have supporting plausible biological hypotheses suggesting causality (See Chapter 2 for detailed discussion on causal mechanisms). Our analysis also showed a statistically significant and robust strength of association and a biological gradient of effect as previously observed in animal studies. Nonetheless, our statistical analysis does not show a temporal association; by this we mean we cannot confirm that metabolic syndrome and hyperuricemia predated the existence of poor collateral status. Reverse causality remains possible. Our analysis is therefore an argument for causality but in itself is not sufficient to prove it. Of note, since we found an independent association of ageing, metabolic syndrome and hyperuricemia with poor collateral status, it is reasonable to assume that these variables are sufficient to cause poor collaterals (if causality is assumed to be valid) but not always necessary. The fact that we did not find any multiplicative interaction amongst these variables in association with poor collateral status supports this argument further. Nonetheless, we will need larger studies, both animal and human, to show consistency of association and to verify causal mechanisms in detail.

Major risk factors associated with stroke occurrence are age, sex, arterial hypertension, cigarette smoking, diabetes mellitus, obesity, physical inactivity, alcohol intake,
psychosocial stress, cardiac disease and dyslipidemia. These risk factors account for up to 90% of the risk of stroke. Many of these same risk factors (age, diabetes mellitus, obesity etc.) are also associated with poor clinical outcome in patients with acute ischemic stroke. Our results also show that some of these same risk factors are also associated with poor collateral status. It is therefore possible that some of these risk factors exert their effect on clinical outcome through poor collaterals while others have an independent effect. Teasing out the potential mediating role of poor collaterals in determining patient clinical outcome is vital. Such information could inform research in the lab on potential therapeutic targets that could be used to augment blood flow through collaterals until recanalization is achieved with thrombolytic agents or intra-arterial removal of clots. Based on results in Chapter 4, a theoretical model that explains how collaterals status mediates the effect of certain risk factors on clinical outcome in patients with acute ischemic stroke is testable using mediational analysis and will serve as the basis for future work.

6.2 Improving collateral imaging

In chapter 5, we demonstrated “effect modification” of the relationship between recanalization (with endovascular therapy) and clinical outcome by collateral status. Patients with good collateral status benefit from recanalization; patients with poor collaterals do not benefit even when recanalization is achieved. In addition, our results support previous literature that suggests that patients with poor collaterals do not do well clinically. We were thus able to show both that collateral imaging using single phase CTA Head examination is a good tool to assess prognosis in ischemic stroke patients with
intracranial occlusions, and it may be used as an imaging selection tool to pick the right patients for this therapy.

Nonetheless, the CTA technique we used in this thesis is a single snap shot of contrast filled blood vessels.\textsuperscript{128} To more accurately measure collateral status, we have developed a new technique; multi-phase CTA that generates time-resolved cerebral angiograms of the brain vasculature from skull base to vertex following contrast injection (Figure 6-1). An ongoing study (PRoveIT) is where we are testing the utility of multi-phase CTA in helping physicians select the right patient for endovascular therapy.

6.3 Genetic determinants of collateral status

Recently, Faber et al. have found that pial collateral extent varies by 56-fold among healthy mouse strains with naturally occurring differences in genetic background.\textsuperscript{5} A single polymorphic locus on chromosome 7, \textit{(Dce1, 734 Kb, 27 SNPs)}, was found to be causal for 83 percent of this variation in collateral extent. Working closely with Dr. Jim Faber (University of North Carolina), a future study will determine if a variant of human \textit{Dce1}, or a related one in the pathway controlling collaterogenesis (eg, \textit{Vegfa, Clic4, Flk1, Dll4}) underlies wide variation in collaterals in acute ischemic stroke patients.
Figure 6-1: Collateral Assessment on Multiphase CTA.

Upper panel shows a patient with a left M1 MCA occlusion (arrow) and good collaterals (backfilling arteries) on multi-phase CTA. Middle panel shows a patient with a left M1 MCA occlusion (arrow) and intermediate collaterals. Lower panel shows a patient with a right M1 MCA occlusion (arrow) and poor collaterals (minimal backfilling arteries) on multi-phase CTA.
REFERENCES


22. Coyle P. Diameter and length changes in cerebral collaterals after middle cerebral artery occlusion in the young rat. The Anatomical record 1984;210:357-64.


98. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before


## APPENDIX 1: STROKE ASSESSMENT SCALES

1a **Level of Consciousness:**
- 0 = Alert, keenly responsive.
- 1 = Not alert, but arousable by minor stimulation to obey, answer, or respond.
- 2 = Not alert, requires repeated stimulation to attend, or is stuporous and requires strong or painful stimulation to make movements (not stereotyped).
- 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, areflexic.

1b **LOC Questions:**
- 0 = Answers both questions correctly
- 1 = Answers one question correctly
- 2 = Answers neither question correctly

1c **LOC Commands:**
- 0 = Performs both tasks correctly
- 1 = Performs one task correctly
- 2 = Performs neither task correctly

2 **Best Gaze:**
- 0 = Normal
- 1 = Partial gaze palsy. This score is given when gaze is abnormal in one or both eyes, but where forced deviation or total gaze paresis are not present.
- 2 = Forced deviation or total gaze paresis is not overcome by the oculocephalic maneuver.

3 **Visual:**
- 0 = No visual loss
- 1 = Partial hemianopia
- 2 = Complete hemianopia
- 3 = Bilateral hemianopia (blind including cortical blindness)

4 **Facial Palsy:**
- 0 = Normal symmetrical movement
- 1 = Minor paralysis (flattened nasolabial fold, asymmetry of smiling)
- 2 = Partial paralysis (total or near total paralysis of lower face)
- 3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)

5 **Motor Arm:**
- 0 = No drift, limb holds 90 (or 45) degrees for full 10 seconds
- 1 = Drift, limb holds 90 (or 45) degrees, but drifts down before full 10 seconds, does not hit bed or other support
- 2 = Some effect against gravity, limb cannot get to or maintain (if used) 90 (or 45) degrees, drifts down to bed, but has some effect against gravity
- 3 = No effect against gravity, limb falls

5a **Left**

5b **Right**

6 **Motor Leg:**
- 0 = No drift, leg holds 30 degrees position for full 5 seconds
- 1 = Drift, leg falls by the end of the 5 second period but does not hit bed
- 2 = Some effect against gravity, leg falls to bed by 5 seconds, but has some effect against gravity
- 3 = No effect against gravity, leg falls to bed immediately

6a **Left**

6b **Right**

7 **Limb Ataxia:**
- 0 = Absent
- 1 = Present in one limb
- 2 = Present in two limbs
- If present, is ataxia in (circle each limb YES or NO)

8 **Sensory:**
- 0 = Normal, no sensory loss
- 1 = Mild to moderate sensory loss
- 2 = Severe to total sensory loss
- Patient is not aware of being touched

9 **Best Language:**
- 0 = No aphasia, normal
- 1 = Mild to moderate aphasia, some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression.
- 2 = Severe aphasia, all communication is through fragmentary expression, great need for inference, questioning, and guessing by the listener.

10 **Dysarthria:**
- 0 = Normal
- 1 = Mild to moderate; patient slurs at least some words and, at worst, can be understood with some difficulty.

11 **Extinction and Inattention (formerly Neglect):**
- 0 = No abnormality
- 1 = Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities
- 2 = Profound hemi-inattention or hemi-extinction to more than one modality. Does not recognize own hand or objects to only one side of space

### National Institutes of Health Stroke Scale (NIHSS)
Structured Interview for the Modified Rankin Scale

5 = Severe disability; someone needs to be available at all times; care may be provided by either a trained or an untrained caregiver. Question: Does the person require constant care?

4 = Moderately severe disability; need for assistance with some basic ADL, but not requiring constant care. Question: Is assistance essential for eating, using the toilet, daily hygiene, or walking?

3 = Moderate disability; need for assistance with some instrumental ADL but not basic ADL. Question: Is assistance essential for preparing a simple meal, doing household chores, looking after money, shopping, or traveling locally?

2 = Slight disability; limitations in participation in usual social roles, but independent for ADL. Questions: Has there been a change in the person’s ability to work or look after others if these were roles before stroke? Has there been a change in the person’s ability to participate in previous social and leisure activities? Has the person had problems with relationships or become isolated?

1 = No significant disability; symptoms present but not other limitations. Question: Does the person have difficulty reading or writing, difficulty speaking or finding the right word, problems with balance or coordination, visual problems, numbness (face, arms, legs, hands, feet), loss of movement (face, arms, legs, hands, feet), difficulty with swallowing, or other symptom resulting from stroke?

0 = No symptoms at all; no limitations and no symptoms.

Modified Rankin Scale with structured interview
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Member, Hotchkiss Brain Institute
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Yours sincerely,

[Signature]

Dr Shelagh B. Coutts BSc, MB, ChB., MD, FRCP, FRCP (Glasg.)
Stroke Neurologist
Alberta Innovates Clinical Investigator and HSFC Distinguished Clinician Scientist.
Associate Professor Department of Clinical Neurosciences and Radiology,
University of Calgary
September 3, 2014

To

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University of Calgary
2500 University Drive NW
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Mohammed A. Ameckhlaifi, MD MSc FRCP
Assistant Professor
Department of Medicine
King Abdulaziz University
Jeddah, Saudi Arabia
September 3, 2014

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Donald G. Welsh, PhD
Professor, University of Calgary
Dept. of Physiology & Pharmacology
September 3, 2014

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Sincerely,

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Tel: (403) 944-8065 Fax: (403) 283-2270
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K.H. Cho
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