Carotid Magnetic Resonance Imaging Depicted Intraplaque Hemorrhage and a High-Risk Cardiovascular and Cerebrovascular Phenotype

By

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Institute of Medical Science
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Abstract

Intraplaque hemorrhage is an independent marker of cardiovascular outcomes including stroke and myocardial infarction. Magnetic resonance imaging allows for the direct visualization of intraplaque hemorrhage in the vessel wall of the carotid arteries. This thesis reports data from an institutional magnetic resonance depicted intraplaque hemorrhage experience and clinical trials. The work herein addresses areas particularly sparse on the relationship of intraplaque hemorrhage with cardiovascular risk factors and its potential role in stroke, specifically reporting on i) age-specific sex differences in low-grade stenosis, ii) the role in ipsilateral embolic stroke of undetermined source, and iii) the relationship with a high-risk cardiovascular phenotype. The results in this thesis support our hypotheses that i) males have greater age-specific odds of magnetic resonance imaging depicted carotid intraplaque hemorrhage compared to females, and that with increasing age, the odds of carotid intraplaque hemorrhage in females becomes closer to that of males, ii) patients with embolic stroke of undetermined source have carotid intraplaque hemorrhage ipsilateral to the affected brain, and iii) patients with carotid intraplaque hemorrhage are of a high-cardiovascular risk. The culmination of projects supports the notion that intraplaque hemorrhage is related to a high-risk cardiovascular phenotype and a need for the quantification
of intraplaque hemorrhage is realized to further understand the significance of volume of intraplaque hemorrhage, longitudinal changes in intraplaque hemorrhage over time, and relationship of intraplaque hemorrhage with clinical outcomes. The final project in this thesis therefore reports the development of a quantitative imaging analysis protocol, as well as its reliability and agreement with an established standard. The protocol is intended for use on carotid trial imaging data presently being acquired as part of the Canadian Atherosclerosis Imaging Network Project 1.
Dedication

In loving memory of my grandfather, Lt. Joginder Singh Baweja (May 26, 1929-June 12, 2016), for taking an interest in the progress of my training upon each meeting, serving as a personal and professional sounding board, and always encouraging me to go one step further.
Acknowledgments

First and foremost, I thank my supervisor, Dr. Alan R. Moody, for his supervision, mentorship, and for piquing my interest in trials of vessel wall magnetic resonance imaging. His work to potentially translate carotid intraplaque hemorrhage magnetic resonance imaging into clinical practice to improve patient outcomes persuaded me to work alongside him for the past decade, first as a medical student starting in 2006 and then via the Clinician Investigator Program’s joint PhD and residency training pathway. I sincerely appreciate the opportunity to participate in his competitively funded clinical imaging trials, including the Canadian Atherosclerosis Imaging Network Project 1. Many lessons were learned including the day-to-day management of a laboratory, obtaining grants, setting up protocols, reviewing images, administration such as reporting incidental imaging findings and collaborating with and mentoring junior group members, and the importance of granular and meticulous data collection to ensure that scientific observations do not go unseen.

Many others have also helped me navigate my studies. My Program Advisory Committee members, including Drs. Subodh Verma, Richard I. Aviv, and Laurent Milot, provided guidance along the way. Drs. Eric Bartlett and Linda Probyn, my current and past residency program directors, supported joint PhD and residency training. My sustained interest in academic medicine is inspired by past and present mentors, and I am grateful to Drs. Gianluca Iacobellis, David J. Gladstone, Sandra E. Black, Andrea Doria, Heather M. Arthur, Bhagu R. Bhavnani, Arya M. Sharma, Del Harnish, Erika Kustra, Anna E. Zavodni, Sean P. Symons, and Allan J. Fox.

To present and past research friends at the Sunnybrook Research Institute and the Vascular Biology Imaging Research Group, thank you for your company and fellowship. My infinitely patient and loving family has provided a vast array of the requisite supports to succeed in completing my training—my parents, Tejinderpal Singh and Manninder Singh, and my siblings, Raviraj Singh, Amandeep Singh, and Harleen Rosie Singh.

No words can convey my love and appreciation to Harleen K. Khanijoun my intelligent, supportive, and insightful partner. I look forward to the next chapter along with our son Ajay and daughter Apar, who have both unwittingly brought perspective and unfettered joy.
Finally, I am grateful to the University of Toronto Diagnostic Radiology Residency Program, Royal College of Physicians and Surgeons of Canada’s Clinician Investigator Program, the Institute of Medical Science Doctor of Philosophy Program, and Cardiovascular Sciences Collaborative Program. Training in these programs has helped me gain skills to advance me toward a career as an independent physician-scientist. The programs have allowed for a multitude of endeavors, a small fraction of which are reflected in this thesis.

Funding acknowledgments for some of the work included in this body of work are 2012-2016 Canadian Institute of Health Research (CIHR) Fellowship in Priority Announcement: Patient-Oriented Research (FRN 120988), 2015 RSNA R&E Research Resident Grant (#RR1561), 2012 RSNA R&E Research Resident Grant (#RR1237), and 2012 Physician Services Incorporated Grant.
Contributions

Navneet Singh was responsible for the preparation of the thesis, including conception, ethics approval, grant funding including CIHR FRN 120988, RSNA #1561, RSNA #1237, and PSI Foundation 2012, data collection, analysis, and writing. The work contained in this thesis was made possible by:

Alan R. Moody, supervisor, for supervision, assistance with all aspects of the thesis, including study conception, direction, peer-review, access to patient data and images, and laboratory resources;

Richard Aviv, Subodh Verma, Laurent Milot, PAC members, for thesis direction and peer review;

Pascal Tyrrell, for database assistance and statistical peer-review of Chapter 2;

Kush Kapur, Alex Kiss, for statistical assistance with Chapters 2 and 4, respectively;

Bowen Zhang, Isabella Kaminski, and Genevieve-Rochon Terry, for assistance with data collection related to Chapters 2 and 4;

David J. Gladstone, for access to clinical trial data and peer review of Chapter 3, and also those who contributed to patient recruitment or data collection at Sunnybrook Health Sciences Centre, Toronto (V. Basile, K. Boyle, J. Hopyan, R. Swartz, H. Vaid, G. Valencia, J. Ween, R. Aviv, S. Symons, A. Fox, P. Howard, R. Yeung), trial design and operations (J. Hall, P. Dorian, M. Spring, M. Mamdani, K. Thorpe, and members of the EMBRACE steering committee, and staff at the Applied Health Research Centre, Li Ka Shing Knowledge Institute of St. Michael’s Hospital, Toronto).

Tishan Maraj, for serving as a second image-rater for Chapter 5; and

Vivek Thayalasuthan, Lena Koh, and Natalie Rashkovan for research assistance including ethics approval.
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# Abbreviations

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<tr>
<td>2D</td>
<td>2-Dimensional</td>
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<tr>
<td>3D</td>
<td>3-Dimensional</td>
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<td>ACAS</td>
<td>Asymptomatic Carotid Atherosclerosis Study</td>
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<td>ACC</td>
<td>American College of Cardiology</td>
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<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
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<td>ACST</td>
<td>Asymptomatic Carotid Surgery Trial</td>
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<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>ARB</td>
<td>Angiotensin Receptor Blocker</td>
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<td>ARIC</td>
<td>The Atherosclerosis Risk in Communities</td>
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<tr>
<td>ASCOD</td>
<td>Atherosclerosis, Small-vessel Disease, Cardiac Pathology, Other Causes, Dissection</td>
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<tr>
<td>B-A</td>
<td>Bland-and-Altman</td>
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<tr>
<td>BB</td>
<td>Black-blood</td>
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<td>CAD</td>
<td>Coronary Artery Disease</td>
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<td>CAIN</td>
<td>Canadian Atherosclerosis Imaging Network</td>
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<td>CCA</td>
<td>Common Carotid Artery</td>
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<td>CD163</td>
<td>Cluster of Differentiation 163</td>
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<tr>
<td>CD68+</td>
<td>Cluster of Differentiation 68</td>
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<tr>
<td>CE</td>
<td>Contrast Enhanced</td>
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<td>CEA</td>
<td>Carotid Endarterectomy</td>
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<td>CI</td>
<td>Confidence Interval</td>
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<td>cIMT</td>
<td>Carotid Intima-media Thickness</td>
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<td>CRP</td>
<td>C-reactive Protein</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<td>CTA</td>
<td>Computed Tomography Angiography</td>
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<td>Cardiovascular</td>
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<td>DSA</td>
<td>Digital Subtraction Angiography</td>
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<td>ECA</td>
<td>External Carotid Artery</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>ECST</td>
<td>European Carotid Surgery Trial</td>
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<td>EMBRACE</td>
<td>Event Monitor Belt for Recording Atrial Fibrillation after a Cerebral Ischemic Event</td>
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<td>ESUS</td>
<td>Embolic Stroke of Undetermined Source</td>
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<td>FLAIR</td>
<td>Fluid-attenuated Inversion Recovery</td>
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<td>FOV</td>
<td>Field of View</td>
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<td>GRE</td>
<td>Gradient Echo</td>
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<td>HIF-1</td>
<td>Hypoxic Inducible Factor 1</td>
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<tr>
<td>HIPPA</td>
<td>Health Insurance Portability and Accountability Act of 1996</td>
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<td>HRCP</td>
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<td>HU</td>
<td>Hounsfield Unit</td>
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<td>ICA</td>
<td>Internal Carotid Artery</td>
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<td>ICC</td>
<td>Intra-class Correlation Coefficient</td>
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<td>Intima-media Thickness</td>
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<td>LR/NC</td>
<td>Lipid Rich-necrotic Core</td>
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<td>MACE</td>
<td>Major Adverse Cardiovascular Events</td>
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<td>MDCTA</td>
<td>Multidetector Computed Tomography Angiography</td>
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<td>MESA</td>
<td>Multi-Ethnic Study of Atherosclerosis</td>
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<td>MI</td>
<td>Myocardial Infarction</td>
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<td>MIP</td>
<td>Maximum Intensity Projection</td>
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<td>MMP</td>
<td>Matrix Metalloproteinase</td>
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<td>MPR</td>
<td>Multiplanar Reformats</td>
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<td>MPRAGE</td>
<td>Magnetization Prepared Rapid Acquisition with Gradient-Echo</td>
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<td>MRA</td>
<td>Magnetic Resonance Angiography</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MR-IPH</td>
<td>Magnetic Resonance Depicted Intraplaque Hemorrhage</td>
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<td>MSDE</td>
<td>Motion Sensitized Driven Equilibrium</td>
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<tr>
<td>NASCET</td>
<td>North American Symptomatic Carotid Endarterectomy Trial</td>
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NAVIGATE | New Approach riVaroxaban Inhibition of Factor Xa in a Global Trial versus Aspirin to Prevent Embolism

OR | Odds Ratio

OW | Outer Wall

PD | Proton Density

PET | Positron Emission Tomography

PVD | Peripheral Vascular Disease

RBC | Red Blood Cell

RCT | Randomized Controlled Trial

RESPECT | Randomized Evaluation of Recurrent Stroke Comparing Patent Foramen Ovale Closure to Established Current Standard of Care Treatment

ROI | Region of Interest

SD | Standard Deviation

SE | Standard Error

SNR | Signal-to-noise Ratio

SPIR | Spectral Pre-saturation with Inversion Recovery

SVD | Small Vessel Disease

T1w | T1-weighted

TCD | Transcranial Doppler

TEE | Trans-esophageal Echocardiography

TIA | Transient Ischemic Attack

TIMP | Tissue Inhibitors of Metalloproteinases

TLR-4 | Toll-like Receptor 4

TOAST | Trial of ORG 10172 in Acute Stroke Treatment

TOF | Time-of-flight

TPA | Total Plaque Area

TPV | Total Plaque Volume

TTE | Transthoracic Echocardiography

VACAS | Veterans Affairs Cooperative Atherosclerosis Study

VEGF | Vascular Endothelial Growth Factor

VW | Vessel Wall
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<td>Vessel Wall Disease</td>
</tr>
<tr>
<td>WMD</td>
<td>White Matter Disease</td>
</tr>
<tr>
<td>WMHI</td>
<td>White Matter Hyperintensity</td>
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</table>
Peer-Reviewed Publications


International Meetings


Chapter 1—Carotid Atherosclerosis Imaging from Lumen to the Vessel Wall

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1.1 Overview

Intraplaque hemorrhage (see Figure 1.1) is an independent marker of cardiovascular outcomes including stroke and myocardial infarction. Magnetic resonance imaging allows for the direct visualization of intraplaque hemorrhage in the vessel wall of the carotid arteries. This thesis reports data from an institutional magnetic resonance depicted intraplaque hemorrhage experience and a clinical trial. The work herein addresses areas particularly sparse regarding the relationship of intraplaque hemorrhage with cardiovascular risk factors and its potential role in stroke, specifically reporting on i) age-specific sex differences in low-grade stenosis, ii) the role in ipsilateral embolic stroke of undetermined source, and iii) the relationship with a high-risk cardiovascular phenotype. This section reviews the literature on the burden, pathophysiology, and imaging of carotid atherosclerosis and culminates in the specific aims and projects presented in the remainder of the thesis.
Figure 1.1: Schematic of Carotid Intraplaque Hemorrhage. The carotid arteries serve as a conduit from the heart to the brain. The presence of a carotid intraplaque hemorrhage is depicted here at the origin of the internal carotid artery. The biomarker provides an opportunity to identify patients who are vulnerable to major adverse cardiovascular events, including stroke and myocardial infarction. Risk factors and consequences of carotid intraplaque hemorrhage are investigated in this body of work using a ten-year institutional experience of intraplaque hemorrhage magnetic resonance imaging. © Navneet Singh, 2016. All rights reserved.
1.2 Cardiovascular Disease Burden

1.2.1 Worldwide

Cardiovascular diseases are a leading cause of mortality and morbidity worldwide (WHO, 2011). Of 57 million total deaths worldwide per annum, 36 million are due to noncommunicable diseases. Of these noncommunicable disease deaths, 17.3 million occur as a result of cardiovascular disease. The distribution of noncommunicable disease mortality worldwide reveals that death from cardiovascular disease exceeds mortality from respiratory illness, cancer, and other noncommunicable diseases (see Figure 1.2). Cardiovascular diseases are the most frequent cause of death worldwide, except in Africa. Noncommunicable diseases may also be outnumbered by communicable diseases in the future.

![Figure 1.2: Distribution of Noncommunicable Disease Mortality Worldwide.](image)

Cardiovascular disease mortality includes myocardial infarction and stroke.

Figure reproduced from the 2011 Global Atlas on Cardiovascular Disease Prevention and Control. The World Health Organization permits reproduction for academic theses.

A closer look at the etiology of worldwide cardiovascular disease mortality reveals that 13.5 of the 17.3 million deaths, or 80%, are directly attributable to atherosclerosis. Atherosclerosis-related myocardial infarction and stroke account for 7.3 and 6.2 million deaths, respectively. The remaining 20% of cardiovascular disease mortality results from congenital
heart disease, rheumatic heart disease, cardiomyopathies, and cardiac arrhythmias. Both sexes are significantly impacted by atherosclerosis-related cerebrovascular disease and ischemic heart disease (see Figure 1.3).

**Figure 1.3:** Distribution of Worldwide Cardiovascular Disease Mortality by Sex and Etiology. Figure reproduced from the 2011 Global Atlas on Cardiovascular Disease Prevention and Control. The World Health Organization permits reproduction for academic theses.

Compared with males, females have a marginally higher mortality from cerebrovascular disease (37 vs. 34%) and a lower mortality from ischemic heart disease (38 vs. 46%) worldwide. The precise reasons for the differences in sex-related mortality from cerebrovascular and ischemic heart disease are topics of ongoing study.

### 1.2.2 North America

Estimates of mortality due to cardiovascular disease burden in North America from the American Heart Association echo the worldwide burden of stroke and myocardial infarction. Mortality estimates suggest that one death from cardiovascular disease occurs every 38.9 seconds in North America. In 2009, the annual age-standardized death rate attributable to all cardiovascular diseases in the United States was 237.1 per 100,000 (Go et al., 2013). Given the United States' population of 307 million in 2009, this rate would translate into 727,913 deaths annually, or nearly 1,994 deaths daily as a result of cardiovascular disease (Bureau, 2009).
Extrapolation of this mortality rate to Canada’s population of 35.7 million in 2015 indicates that 84,762 deaths occur per annum, or 232 Canadians die each day as a consequence of cardiovascular disease (Statistics-Canada, 2015).

Atherosclerosis-related myocardial infarction accounts for 20.3% of all deaths in the United States (Marczak, O’Rourke, Shepard, for the Institute for Health, & Evaluation, 2016) and is the leading cause of death in both men and women. Myocardial infarction, stroke, and other cardiovascular causes of mortality are estimated to be 116.1, 38.9, and 81.0 per 100,000 persons in the United States per annum, respectively (Go et al., 2013). Although myocardial infarction is responsible for more mortality than stroke, the American Heart Association estimates that 7,000,000 Americans over the age of 20 have had a stroke (Roger et al., 2012). Annually, 795,000 Americans experience a new or recurrent stroke (Roger et al., 2012). Improvements in mortality from stroke over the past few decades are attributed, in part, to the presence of regional stroke centers (Barnett & Buchan, 2000). Further improvements are likely to evolve given that recent trials have demonstrated the benefit of intra-arterial therapy (Berkhemer et al., 2015) for acute stroke. Currently, however, stroke is responsible for approximately 1 out of every 18 deaths in the United States (Roger et al., 2012).

Stroke accounts for 1.7% of all health expenditures in the United States and is expected to increase by 129% to nearly 240 billion by 2030 compared with 2010 (Ovbiagele et al., 2013). By 2030, the total direct costs are estimated to increase to $184.13 billion from $71.55 billion. Indirect costs such as loss of productivity are expected to rise to $56.54 billion, representing an increase of nearly $23 billion.

Cardiovascular disease accounts for 17% of all health care expenditures in the United States and is expected to increase by 61% to $1.094 trillion dollars by 2030 compared with 2010 (P. A. Heidenreich et al., 2011). By 2030, the total direct costs are estimated to increase to $818 billion from $273 billion (see Figure 1.4). Indirect costs such as loss of productivity are expected to rise to $276 billion, representing an increase of nearly $100 billion (see Figure 1.4).
Forecasts from various organizations on the burden and cost of myocardial infarction and stroke, including the World Health Organization, the American Heart Association, and the American Stroke Association, suggest a significant and persistent burden of cardiovascular disease. Additional cardiovascular disease prevention strategies seem to be needed to address the burden of atherosclerosis.

1.3 Atherosclerosis

1.3.1 Pathological Stages of the Disease

Atherosclerosis is the underlying pathological state responsible for the majority of the cardiovascular disease burden worldwide. The Greek origin of the word *atherosclerosis* describes the pathological changes of a diseased vessel wall. The words *athero* and *sclerosis* transliterate to *gruel* and *hardening*, respectively. Gruel, or various cellular debris, accumulates in the vessel wall during more complicated stages of the disease associated with artery hardening or stiffening compared with a healthy artery (Selwaness, van den Bouwhuijsen, Mattace-Raso, et al., 2014).

The healthy vessel contains three layers. The first layer abutting the lumen is the tunica intima and is comprised of a monolayer of endothelial cells that contact the blood. The intimal
layer contains smooth muscle cells in humans and is lined by a basement membrane. Second, the medial layer is comprised of an extracellular matrix containing smooth muscle cells. Layers of elastin separate the smooth muscle cells to ensure the elastic potential of the arteries required for vasoactivity. Finally, the adventitial layer is comprised of mast cells, nerve endings, and microvessels.

Figure 1.5 summarizes the stages of atherosclerosis from a healthy vessel to (a) intimal thickening with macrophage accumulation, (b) attempted reparative stabilization with smooth muscle cells and neovascularization, (c) and the ultimate consequences of atherosclerosis, including luminal narrowing, plaque fibrous cap rupture, and/or intraluminal thrombosis (d).
The formation of atherosclerotic plaque, a local manifestation of the systemic disease in atherosclerosis (Tomey, Narula, & Kovacic, 2014), is initiated by several factors that disturb endothelial function. These factors include aging, hyperglycemia, hypercholesterolemia, hypertension, male gender, and smoking (Michel, Virmani, Arbustini, & Pasterkamp, 2011). Endothelial dysfunction results in a loss of normal anti-inflammatory, anti-thrombotic, and vasoactive functions (Cahill & Redmond, 2013). Endothelial dysfunction occurs due to the inadequate bioavailability of nitrous oxide, a molecule that is important for the local regulation of vascular tone. Nitric oxide is produced by the enzyme nitric oxide synthase in endothelial cells. Factors such as female sex may be protective against atherosclerosis due to the ability of estrogen to upregulate nitric oxide synthase expression and the production of nitric oxide.

Normal cholesterol handling at the interface between the vessel wall and the lumen is disturbed such that there is a net ingress of cholesterol into the intima and increased cholesterol deposition. Over time, the cholesterol coalesces into lipid pools. This intraplaque environment results in lipid oxidation and the production of oxygen free radicals.

In response to early atherosclerotic changes in the vessel wall, circulating monocytes are attracted within the plaque through endothelial activation and the expression of surface receptors. Factors including dyslipidemia and hypertension may increase monocyte adhesion to the endothelium. Figure 1.6 depicts, along with corresponding changes in macrophages, the evolution of atherosclerosis from pathological intimal thickening to the development of a thin cap fibroatheroma. Cluster of Differentiation 68 (CD68+), a glycoprotein that binds to low-density lipoprotein, is expressed on the surface of monocytes and macrophages. The expression of CD68+ appears dark brown after immunohistochemical processing. During pathologic intimal thickening (see Figure 1.6a, panel 1), lipid pools may accumulate in the deeper part of the intima (see Figure 1.6a, panel 2). Macrophages are present near the surface of the intima. As the lipid pool increases in size into an early-stage necrotic core (see Figure 1.6b, panel 2), macrophages infiltrate the core in an attempt to clear it. Macrophages are shown infiltrating the early necrotic core (see Figure 1.6b, panel 2).

Phagocytosis of plaque lipids by macrophages results in foam cell formation commonly leading to macrophage apoptosis rather than the removal lipids from the vessel wall. Thus, the
fibroatheroma with a late necrotic core has a lytic appearance and a larger size (see Figure 1.6c, panel 1). A reparative response to disease within the plaque results in stimulation of smooth muscle cell proliferation and development of intima-medial thickening in an attempt to stabilize the inflammatory process. A thin fibrous cap characterizes the progression of the disease to the more advanced fibroatheroma (see Figure 1.6d). The thin fibrous cap is prone to rupture and results in intraluminal thrombosis.

**Figure 1.6:** Pathology of Atherosclerosis Progression from Intimal Thickening to Advanced Thin Cap Fibroatheroma. (A) Pathologic intimal thickening, (B) fibroatheroma with an early necrotic core, (C) fibroatheroma with a late necrotic core, and (D) Thin-cap fibroatheroma. The coronary pathology is shown. Reproduced with permission from (Fleg et al., 2012) (License 3800431075269).

### 1.3.2 Classification

Atherosclerotic lesions are commonly classified using definitions based on histology from Stary et al. (see Table 1.1) (Stary et al., 1995). Observations of pathology in the coronary arteries inform the classification system. In particular, Type VI plaques are considered complicated plaques that are vulnerable to rupture and/or thromboembolism. Hemorrhage in the vessel wall
plaque resulting from leaky neoadventitia originating in the vasa vasorum is another feature of a Type VI plaque. Several studies use this classification system to describe advanced stages of atherosclerosis in the carotid arteries.
### Table 1.1: Lesion Nomenclature and Histological Features along with a Cross-sectional Sketch of the Stary Classification of Atherosclerosis. Adapted from (Stary et al., 1995) with permission from Wolters Kluwer Health, Inc. (License 3793710463590).

<table>
<thead>
<tr>
<th>Lesion Nomenclature</th>
<th>Histological Features</th>
<th>Cross-sectional Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I—Initial</td>
<td>Isolated macrophage foam cells</td>
<td>--</td>
</tr>
<tr>
<td>Type II—Fatty Streak</td>
<td>Intracellular lipid accumulation</td>
<td>Type II lesion</td>
</tr>
<tr>
<td>Type III—Intermediate</td>
<td>Intracellular and extracellular lipid pools</td>
<td>Type III (atheroma)</td>
</tr>
<tr>
<td>Type IV—Atheroma</td>
<td>Extracellular lipid core</td>
<td>Type IV (atheroma)</td>
</tr>
<tr>
<td>Type V—Fibroatheroma</td>
<td>Lipid core(s) and fibrotic layer(s), or mainly calcific, or primarily fibrotic</td>
<td>Type V (fibroatheroma)</td>
</tr>
<tr>
<td>Type VI—Complicated</td>
<td>Surface defect, hematoma-hemorrhage, thrombus</td>
<td>Type VI (complicated lesion)</td>
</tr>
</tbody>
</table>
1.3.3 Progression

The progression of disease may be accelerated as a result of a hypoxic intraplaque environment leading to the development of leaky young vasa vasorum, which enhances the development of the necrotic core. Specifically, an indirect effect of plaque surface thickening is the loss of the usual oxygen diffusion from the lumen into the vessel wall once the thickening exceeds 200 microns. In response to this phenomenon and secondary to the release of factors such as HIF-1 (hypoxic inducible factor-1) and the presence of intraplaque macrophages, VEGF (vascular endothelial growth factor) is released. These processes stimulate new vessel growth within the plaque, predominantly originating from the vasa vasorum within the adventitia. The new vessel growth, however, results in leaky, friable vessels that are vulnerable to red cell deposition and frank hemorrhage within the vessel wall (Virmani, Kolodgie, Burke, et al., 2005). The trigger for plaque hemorrhage is not clearly understood, but plaque morphology, hemodynamics, or changes in systemic blood pressure, particularly changes in pulse pressure (Selwaness et al., 2013), may result in repeated microhemorrhages within the plaque. Low diastolic pressure has also been hypothesized in exploratory work to be associated with the presence of intraplaque hemorrhage (Sun et al., 2016). Low diastolic pressure may reflect an acute change in hydrostatic pressure resulting in a pressure gradient from the high-pressure vasa vasorum environment to the low-pressure intraplaque environment.

The influx of red blood cells within the plaque leads to some deleterious effects. First, if the volume of blood deposited in the atherosclerotic plaque is large, it can result in a significant change in the vessel wall volume and potentially encroach on the lumen. The deposition of red blood cells is considered one of the causes of rapid changes in stenosis; a rapid reduction can also occur with blood resorption. More common are repeated small hemorrhages that do not cause a significant change in plaque volume. These hemorrhages result in the repeated delivery of red cells deep within the vessel wall lesion. There are two significant and related effects of repeated red blood cell delivery. Red blood cell membranes have one of the highest cholesterol contents of any cell in the human body, which is further increased during states of hypercholesterolemia. Consequently, each red blood cell delivered into the plaque adds to the
lipid core. Following red blood cell lysis, the other major component that is directly introduced into the plaque is hemoglobin.

Hemoglobin is bound to haptoglobin so that it may be taken up by macrophages via the Cluster of Differentiation 163 (CD 163) receptor (see Figure 1.7). Hemoglobin is broken down into heme and globin. Globin is further broken down into amino acids that are repurposed. Heme is highly inflammatory, and through the Fenton reaction, drives the repetitive production of oxygen free radicals, which contribute to the hostile plaque environment. Heme itself is broken down by heme-oxygenase, resulting in iron and biliverdin. Biliverdin is converted into bilirubin and transported by albumin to the liver, where it is conjugated and excreted into the intestines as bile. Some of the bilirubin derivatives are absorbed into the blood from the intestines and are excreted in the urine. Free iron in macrophages is bound to the protein ferritin and transported via transferrin to the liver or the spleen for storage or to the bone marrow for the generation of new red blood cells. Excess iron in macrophages is problematic for several reasons. First, it may result in increased reactive oxygen species generation through the Fenton reaction. Second, the inability to remove free iron may be associated with the failure to efflux cholesterol from macrophages via ABCA1 (ATP-binding cassette transporter-1). Third, the inability to excrete iron may activate the TLR-4 (Toll-like Receptor 4) signaling pathway. The processes involved in lowering intracellular iron are also associated with increased expression of HIF-1 and VEGF, which may contribute to angiogenesis.
One of the key studies describing the contribution of red blood cells to the atherogenic stimulus was reported in 2003. Kolodgie et al. suggested that erythrocyte membranes are a potent and specific atherogenic stimulus. Glycophorin A is a glycoprotein located on the red blood cell (RBC) membrane that is rich in sialic acid, allowing RBCs hydrophilicity and circulation without adhering to the vessel wall. A small amount of glycophorin A and iron has been detected in earlier stages of atherosclerosis. In contrast, large amounts of glycophorin A and iron were found in later stages of atherosclerosis. Figure 1.8 demonstrates some of the findings associated with fibroatheroma with late stage necrosis (see Figure 1.8, a-e) and a thin fibrous cap (see Figure 1.8, f-j). The fibroatheroma with late stage necrosis is stained darkly for macrophages (see Figure 1.8b), glycophorin A (see Figure 1.8c), iron (see Figure 1.8d), and new vessels with perivascular von Willebrand factor (vWF), indicating the presence of leaky vessels (see Figure 1.8e). The presumably later stage fibrous cap atheroma has a thinner fibrous cap (see Figure 1.8f), positive staining for macrophages (see Figure 1.8g), glycophorin (see Figure 1.8h), and iron (see Figure
1.8i), and darker staining associated with new vessels that are positive for vWF expression, indicating the presence of leaky vessels.

Figure 1.8: Pathology of Fibroatheromatous Plaque. Intraplaque Hemorrhage in Fibroatheroma with a Core in the Late Stage of Necrosis (panels a-e) and Thin-Cap Fibroatheroma (panels f-j). Low power views of the necrotic core used Movat’s Pentachrome at x20 magnification (panels a and f), macrophage immunostaining for CD68+ with x200 magnification (panels b and g), glycophorin A staining at x200 magnification (panels c and h), iron deposits shown in blue stained with Mallory’s at x200 magnification (panels d and i), and vasa vasorum and von Willebrand factor staining at x500 magnification (panels e and j). Reproduced with permission from (Kolodgie et al., 2003), © Massachusetts Medical Society.

The combination of cholesterol and heme delivered with every red cell therefore provides an inflammatory combination that drives the atherosclerotic process. Perpetual monocyte/macrophage migration into the plaque results in increased expression of inflammatory cytokines, enhanced angiogenesis, lipid oxidation, and cell death, resulting in enlargement of the necrotic core of the plaque, neovascularization, and subsequent hemorrhage. This vicious cycle likely accounts for the acceleration in atherosclerotic disease from a slow, predictable progression to more rapid, unpredictable plaque progression and disruption. The presence of plaque hemorrhage therefore denotes a more advanced atherosclerotic state consistent with the increased plaque vulnerability and plaque progression. The role of intraplaque hemorrhage (IPH)
in clinically relevant carotid artery disease progression is one feature of atherosclerosis that is addressed in this thesis.

1.3.4 End-Organ Outcomes

The progression of atherosclerosis into a later disease state may result in luminal narrowing or plaque rupture. The carotid artery is used to highlight the principles of end-organ outcomes related to atherosclerosis in this section. Brott et al. summarize at least five mechanisms that are thought to be implicated in end-organ outcomes (Brott et al., 2011). First, artery-to-artery embolism may occur as a result of the formation of a thrombus on the luminal surface of the atherosclerotic plaque in the carotid artery. Subsequent downstream occlusion of a smaller artery due to this thrombus may occur. Second, thromboembolism of cholesterol crystals or other debris may occur. Third, plaque rupture may result in an acute thrombus, causing local occlusion. Fourth, the presence of carotid atherosclerosis may lead to arterial wall ulceration at the luminal surface of a plaque, resulting in dissection or sub-intimal hematoma. Finally, reduced perfusion may occur due to higher grades of stenosis resulting from progressive plaque growth. Typically, this mechanism requires compromised intracranial collateral circulation.

Regardless of the exact underlying mechanism, both luminal narrowing and plaque rupture with subsequent thromboembolism are implicated in end-organ outcomes of atherosclerosis such as infarction. The latter mechanism is more likely to be involved in end-organ outcomes because high degrees of carotid stenosis, on the order of 60% to 75%, are required to result in significant decreases in cerebral blood flow in-vivo. At first glance, this may appear counterintuitive; considering the carotid artery in isolation in an ideal setting, it would appear that luminal narrowing has the most profound effect on blood flow. The effect of luminal narrowing on blood flow in an artery is dependent on the resistance (R) of liquid in a tube (Klabunde, 2012). Resistance is affected by the tube length (L), viscosity (n), and tube radius (r), among which the radius appears to exert the greatest impact:

\[ R \propto \frac{n \cdot L}{r^4} \]

Flow (Q) is affected by perfusion pressure (\(\Delta P\)) and resistance (R):
\[ Q \propto \frac{\Delta P}{R} \]

A combination of these equations for flow and resistance provides Poiseuille's equation, which summarizes the relationship of flow with perfusion pressure, radius, length, and viscosity:

\[ Q = \frac{\pi \Delta P \cdot r^4}{8n \cdot L} \]

As observed for resistance, the effect of radius on flow is exponential. The reduction in the radius of an artery or arterial stenosis should have the largest quantitative impact on flow among factors such as tube length, blood viscosity, and perfusion pressure.

However, several other factors must be considered \textit{in-vivo} because the vessels are not straight, blood is not a Newtonian fluid, blood may not flow in a laminar fashion, and most importantly, the large carotid artery only accounts for a small portion of the resistance in the neurovascular system. Carotid stenosis is much less likely to affect flow until approximately 60% to 75% stenosis is reached because of the aforementioned features and because the large carotid artery accounts for a small part of the overall resistance in the neurovascular system.

To better understand the impact of the carotid artery as one factor that contributes to neurovascular resistance, consider the following example. Given that the mean carotid artery radius is in the range of 2.5 mm (Krejza et al., 2006), a 50% stenosis of the artery would indicate a reduction in the radius of approximately 1.25 mm. Assuming that all other variables are kept constant and assuming a straight tube, applying Poiseuille's equation shows an anticipated flow rate reduction from 15.3 to .96 (or 16-fold):

\[ Q_{\text{no stenosis}} = \frac{\pi \Delta P \cdot r^4}{8n \cdot L} = \frac{3.14 \cdot 1 \cdot 2.5^4}{8 \cdot 1 \cdot 1} = 15.3 \]

\[ Q_{50\% \text{ stenosis}} = \frac{\pi \Delta P \cdot r^4}{8n \cdot L} = \frac{3.14 \cdot 1 \cdot 1.25^4}{8 \cdot 1 \cdot 1} = 0.96 \]

Therefore, assuming a total neurovascular system resistance of 100, a stenosis of 50% should increase the resistance to 1600. However, to maintain cerebral blood flow, there is a progressive recruitment of cerebral collaterals as a result of cerebral autoregulation that results in the dilation of resistance vessels (Fisch & Brown, 2016). The carotid artery resistance only accounts for 1% of the total resistance of the neurovascular system (Klabunde, 2012), and therefore only an increase in resistance of 16% occurs. The flow from the carotid accounts for
only a small amount of the resistance of the entire neurovascular system. The neurovascular system is comprised of other vessels, either connected in parallel such as the contralateral carotid or vertebrobasilar arteries or connected in series such as the smaller arteries and arterioles that account for much of the resistance. An early report in a series of 17 patients with stenosis undergoing carotid endarterectomy reported the mean internal carotid flow and regional cerebral blood flow using a 133 Xenon injection technique (Boysen, Ladegaard-Pedersen, Valentin, & Engell, 1970). Upon completion of the endarterectomy, the mean flow in the internal carotid artery increased from 133 to 212 ml/min. However, the cerebral blood flow remained unchanged from the preoperative values. Thus, even in patients with stenosis requiring endarterectomy, cerebral autoregulatory mechanisms appear to be able to preserve cerebral blood flow. The autoregulation curve of Lassen (see Figure 1.9) demonstrates the intrinsic ability of the brain to maintain a stable blood flow despite fluctuations in perfusion pressure (Budohoski et al., 2013).

**Figure 1.9:** Relationships among Cerebral Blood Flow, Cerebral Reactivity, Vessel Diameter, and Cerebral Perfusion Pressure. Autoregulation is essentially the ability of the vessel to adapt to changes in cerebral perfusion pressure to maintain blood flow. Reproduced with permission from (Budohoski et al., 2013) (Nature Publishing Group, License 3800990878837).

This Lassen curve was generated using a thermal diffusion regional cerebral blood flow monitoring system and demonstrates the relationships among cerebral blood flow, cerebral
perfusion pressure, cerebral vessel reactivity, and vessel diameter (Budohoski et al., 2013). In the normal range of autoregulation, as cerebral perfusion pressure increases, the vascular diameter decreases and cerebrovascular reactivity increases, allowing for the maintenance of cerebral blood flow. Outside the normal range, cerebral blood flow is increased when above the upper limit of normal autoregulation, and is decreased when below the limit of normal autoregulation. Normal ranges are defined as cerebral perfusion pressure of 62 to 88 mmHg, cerebral blood flow of approximately 27 ml/min/100 g, and cerebrovascular reactivity of 2 to 3 mmHg/ml/min/100 g).

Cerebral ischemia occurs as a result of a reduction in cerebral blood flow. The cerebral blood flow continuum encompasses ischemia (<20 ml/100 g/min), oligemia (20 to 40 ml/100 g/min), normal (40 to 60 ml/100 g/min), and hyperperfusion (>60 ml/100 g/min). Within the ischemic continuum, the infarction threshold is <8 ml/100 g/min. Critical and penumbra thresholds are 12 and 20 ml/100 g/min, respectively.

Cerebral ischemia resulting from hemodynamic compromise therefore requires a significant reduction in the carotid artery lumen and impaired autoregulation (Schoof et al., 2007; White & Markus, 1997) or poor intracerebral vessel collateralization. Another hemodynamic issue arises as a result of the natural history of carotid atherosclerosis progression and the positive remodeling that takes place in the early phases of atherosclerosis. The substantial burden of carotid artery atherosclerosis may exist before any significant reduction in the lumen. Observations from Glagov, commonly referred to as the Glagov phenomenon, suggest that stenosis alone does not accurately reflect atherosclerotic burden (S. Glagov et al., 1987). Morphological changes in atherosclerosis begin with an outward expansion of the vessel. Glagov demonstrated this in early pathological specimens of the left main coronary arteries (see Figure 1.10).
Figure 1.10: Natural History of Atherosclerosis and Positive Remodeling of the Vessel in Early Stages of Disease. The lumen initially enlarges up to 40% stenosis, at which point plaque accumulation occurs at a higher rate, resulting in luminal narrowing. Reproduced with permission from (Glagov, Weisenberg, Zarins, Stankunavicius, & Kolettis, 1987), © Massachusetts Medical Society.

More recently, positive remodeling in the carotid arteries was preliminarily reported in 201 individuals with a low-moderate Framingham-risk score using CT angiography (CTA) and magnetic resonance imaging (MRI). Intra-individual patient correlation between remodeling in early atherosclerosis was found in left and right carotid arteries. Coronary arteries, however, did not appear to correlate well with carotid artery remodeling ($r=0.20$, $p<0.0001$). These findings suggest that there are differences in remodeling between the coronaries and the carotids. However, both undergo similar remodeling in early stages of disease (Selwaness & Bluemke, 2015). The finding that carotid remodeling is not correlated with coronary remodeling also may explain why carotid stenosis has limited utility for the identification of patients at risk of ischemic coronary events.

Several issues surround the use of carotid stenosis for the identification of patients at risk of cerebral ischemia. In patients with impaired cerebrovascular regulation, carotid artery stenosis may not necessarily result in ischemia until higher grades of stenosis occur, and it may not represent an atherosclerotic burden in the vessel wall (see Figure 1.11). These issues are among those hypothesized to explain the reason that the degree of stenosis is unable to accurately identify individuals at risk of ischemic cerebrovascular events. For example, among symptomatic patients with severe stenosis, the number needed to treat to prevent one stroke two years after
carotid endarterectomy is eight patients (Gorelick, 1999). For symptomatic moderate and low-grade carotid stenosis, the numbers needed to treat are as high as 20 and 67 patients, respectively. Asymptomatic patients with greater than 50% to 60% stenosis require as many as 48 to 83 patients to prevent one stroke. Better indicators than luminal stenosis for the identification of patients at risk of stroke are clearly necessary.

Figure 1.11: Progression of Atherosclerosis, Stenosis, and Vessel Wall Plaque Components. This figure shows the progression of atherosclerosis and demonstrates positive remodeling in early stages of atherosclerosis. Encroachment of the lumen does not occur until later stages of the disease. Evaluating the lumen may therefore underestimate the amount of true plaque burden. Plaque components may not be assessed with traditional imaging methods focusing on the lumen. Evaluation of the vessel wall may provide improved quantification and characterization of atherosclerosis disease burden. © Navneet Singh, 2016. All rights reserved.

Given that stenosis may not accurately reflect the underlying atherosclerosis burden and the corresponding stroke risk, plaque components and vessel wall burden have become targets of study. Thromboembolism related to advanced or complicated plaque characteristics, such as IPH, is emerging as a leading mechanism of atherosclerosis related end-organ outcomes. Downstream blockages from thromboembolism of smaller resistance arteries may impair blood flow and result in ischemia. While some studies have suggested that plaque components directly affect flow, it is unclear if plaque components can overcome the cerebrovascular hemodynamic equilibrium to the point of ischemia. For example, IPH is implicated in the rapid expansion of
plagues potentially resulting in critical stenosis (Beach et al., 1993). More recently, the presence of IPH has been implicated in affecting cerebral blood flow reduction, in addition to and independent of stenosis (Hashimoto, Hama, Yamane, & Kurisu, 2013). However, based on opportunities for cerebral collateralization, autoregulation, and the requirement for very high grades of stenosis to reach a critical point, the instability of plaque components is a leading reason for end-organ outcomes related to atherosclerotic plaque components.

Plaque components may also play a role in inducing vulnerability and rupture. Rupture of the fibrous cap or ulceration of the plaque may result in the contact of intravascular blood with a plaque. Platelets and coagulation proteins in the blood contacting various components of the plaque, including tissue factors and collagen, can promote thrombosis (Brott et al., 2011). Several morphological characteristics, such as the presence of IPH, a thin fibrous cap, and ulceration, are also associated with plaque rupture (Hermus, Lefrandt, Tio, Breek, & Zeebregts, 2010) (see Table 1.2). These plaque components may also be associated with processes that contribute to vulnerability, including inflammation, lipid accumulation, apoptosis, proteolysis, thrombosis, and angiogenesis (Hermus et al., 2010). Plaque components themselves are not necessarily indicated in expansive remodeling, and therefore, strategies for the direct visualization of these components may be useful (Saam et al., 2016).

Table 1.2: Morphological Features Related to Plaque Instability. Reproduced from (Hermus et al., 2010) with permission from Elsevier (License 3801400842160).

<table>
<thead>
<tr>
<th>Unstable plaques</th>
<th>Stable plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atheromatous</td>
<td>Fibrous</td>
</tr>
<tr>
<td>Thin fibrous cap</td>
<td>Thick fibrous cap</td>
</tr>
<tr>
<td>Large lipid core</td>
<td>Small lipid core</td>
</tr>
<tr>
<td>Less collagen</td>
<td>Collagen-rich</td>
</tr>
<tr>
<td>Noncalcified</td>
<td>Calcified</td>
</tr>
<tr>
<td>Ulceration</td>
<td>No ulceration</td>
</tr>
<tr>
<td>Intraplaque hemorrhage</td>
<td>No intraplaque hemorrhage</td>
</tr>
<tr>
<td>Infiltration of inflammatory cells (macrophages)</td>
<td>No infiltration of inflammatory cells</td>
</tr>
<tr>
<td>Proteolysis and remodeling</td>
<td>No remodeling</td>
</tr>
</tbody>
</table>
Vulnerable plaques may be transient, and the identification of patients who are unable to undergo repair of higher-risk plaques—that is, high-risk patients, may be more important. Managing patients at risk of cardiovascular outcomes is a greater priority than managing individual plaques (Libby & Pasterkamp, 2015). The identification of a vulnerable plaque, however, may prompt a search for the presence of a systemic disease or a high-risk cardiovascular phenotype and allow for identification of patients at risk of cardiovascular events. The characterization of plaque components may permit the identification of a cardiovascular phenotype. Characterization may also provide greater insights into patients who are prone to systemic atherosclerosis and associated complications.

1.4 Carotid Artery Atherosclerosis

Evaluation of the carotid arteries, the conduits between the heart and brain, provides an opportunity to identify “vulnerable plaques” that are at risk of rupture and progression and "vulnerable patients" who are at risk of stroke and myocardial infarction. This section reviews the anatomy of the carotid artery and discusses anatomical site predilection to atherosclerosis. The role of carotid atherosclerosis in ischemic stroke and the relationship of atherosclerosis with systemic or polyvascular disease such as ischemic heart disease are discussed. The functions of both contemporary and advanced carotid artery imaging in cardiovascular disease prevention are emphasized in subsequent sections.

1.4.1 Anatomy

Carotid arteries at the aortic arch are subject to considerable variation. In 70% of cases, the right and left carotid artery originate from the brachiocephalic (innominate) artery and aortic arch, respectively (Layton, Kallmes, Cloft, Lindell, & Cox, 2006) (see Figure 1.12).
Figure 1.12: Carotid Origin and the Aortic Arch. The most common aortic arch branching pattern in humans has separate origins for the innominate, left common carotid, and left subclavian arteries. Reproduced with permission from (Layton et al., 2006) (License: 3850340297028).

Other common variants of the arch exist, including arch origination of i) the left common carotid artery from the innominate artery found in 13% of patients (see Figure 1.13), and ii) the left common carotid artery from an innominate artery found in 9% of patients (see Figure 1.13). These two variations are two to three times more likely to be found in African Americans than in Caucasian individuals.

These common variants are sometimes erroneously referred to as bovine arches. A true bovine arch, however, is characterized by a single brachiocephalic trunk from the aortic arch that splits into bicarotid and bisubclavian arteries and is not common (see Figure 1.14). The bovine arch is one of more than 20 arch variants.

**Figure 1.14:** True Bovine Arch. Reproduced with permission from (Layton et al., 2006) (License: 3850340297028).

As the carotid arteries course through the neck, they bifurcate into the internal and external branches at the thyroid cartilage. The right and left carotid bifurcations are usually within 5 cm of each other (Brott et al., 2011). The internal carotid is dilated at the origin, and beyond this bulb, the internal carotid artery walls become parallel. The internal carotid artery does not have any extracranial branch vessels, unlike the external carotid artery. The external carotid artery branches to supply the face. The extracranial external carotid artery also provides collateral flow to the internal carotid artery via several pathways (Liebeskind, 2003; Shuaib, Butcher, Mohammad, Saqqur, & Liebeskind) (see Figure 1.15).
Figure 1.15: External Carotid Artery Collateral Arterial Anastomoses. From the facial (a), maxillary (b), and middle meningeal (c) arteries to the ophthalmic artery and dural arteriolar anastomoses from the middle meningeal artery (d) and occipital artery through the mastoid foramen (e) and parietal foramen (f). From (Liebeskind, 2003) (License 380205711023).

The internal carotid artery is comprised of seven segments, C1 to C7, per traditional nomenclature (see Figure 1.16). The cervical segment (C1) courses through the neck from the carotid bifurcation and does not contain any branches. The petrous segment (C2) is fixed to the bone as it enters the skull base and is commonly cited as the reason that dissections do not extend intracranially. The lacerum (C3) is the segment traversing the skull base foramen lacerum. The cavernous (C4) segment is the portion of the carotid that courses through the cavernous sinus. The C4 segment gives rise to the meningohypophyseal trunk, which supplies the pituitary, tentorium, and dura of the clivus. The C4 segment also gives rise to the inferolateral trunk to supply the third, fourth, and sixth nerve as well as the trigeminal ganglion. The C5, or clinoid segment, is delineated by two rings that mark the proximal and distal portions of the clinoid segment. The distal ring may prevent intracranial subarachnoid hemorrhage with rupture of a clinoid segment aneurysm. The supraclinoid region (proximal C6, referred to as ophthalmic, and distal C7, referred to as communicating) gives rise to several important branches. These branches include the ophthalmic artery, which supplies the optic nerve, the posterior communicating artery
that anastomoses with the posterior circulation (if it supplies the entire posterior circulation via an enlarged posterior communicating artery, it is referred to as a fetal posterior cerebral artery), and the anterior choroidal artery that supplies the optic chiasm, hippocampus, and posterior limb of the internal capsule.

Figure 1.16: Internal Carotid Artery Segments. Key landmarks defining each of the segments from C1 through C7 are shown. C1 = Cervical segment, C2 = Petrous segment, C3 = Lacerum segment, C4 = Cavernous segment, C5 = Clinoid segment, C6 = Ophthalmic segment, C7 = Communicating segment. © Navneet Singh, 2016. All rights reserved.

1.4.2 Anatomical Site Predilection

Atherosclerosis has a predilection for the extracranial carotid bifurcation and other branch points despite the exposure of the entire arterial tree to cardiovascular risk factors (Morbiducci et al., 2016). Atherosclerosis progresses at higher rates in the internal carotid artery than the common carotid artery, and disease in the internal carotid artery appears to more accurately correlate with
a patient’s clinical vascular risk factors (Mackinnon et al., 2004). The internal carotid artery is located at a junction point, supplying the low-pressure cerebral circulation; the external carotid branch provides blood to the high-resistance facial muscles. Hemodynamic disturbances, such as low shear stress and a high oscillatory shear index, localized to these branch points and, in particular, the bulb region of the internal carotid and common carotid regions (Markl et al., 2010), are thought to be associated with atherosclerotic plaque progression and composition. These particular regions have a predilection for atherosclerosis because they are subject to low or oscillatory endothelial shear stress or are located near branch points (Bentzon, Otsuka, Virmani, & Falk, 2014). Low shear stress is associated with disturbed flow, whereas high shear stress is associated with normal laminar flow (Morbiducci et al., 2016). Reports have suggested that low shear stress is involved in intimal thickening in hypertensive patients (Jiang, Kohara, & Hiwada, 1999). The role of shear stress in the pathogenesis of atherosclerosis may be related to endothelial dysfunction due to decreased nitrous oxide production from endothelial nitrous oxide synthase (Cahill & Redmond, 2013), which is discussed further here (Cunningham & Gotlieb, 2004; Morbiducci et al., 2016). Low shear stress surrogates, such as geometry (Morbiducci et al., 2016), have also been implicated in the development of atherosclerosis (Phan et al., 2012). The internal carotid artery angle of origin has been shown to be associated with atherosclerosis (Sitzer et al., 2003). Ischemic stroke is more often diagnosed in the left compared with the right hemisphere, and the underlying etiology of this discrepancy is unclear. Recently, differences in plaque components have been described, with a greater prevalence of unstable plaque features in the left versus the right carotid in the Rotterdam cohort (Selwaness, van den Bouwhuijsen, van Onkelen, et al., 2014). The underlying reason for this predilection may be due to differences in shear stress between the left and right carotid arteries due to the arterial anatomy. Blood flow to the right carotid artery is more direct, presumably resulting in higher shear stress. In contrast, by the time blood reaches the left internal carotid artery, it has travelled a longer path and has curved along the aortic arch. Presumably, any presence of atherosclerosis along the way could induce lower shear stress. While the increased diagnosis of stroke in the left hemisphere may be related to the underdiagnosis of right hemisphere stroke in clinical practice (Paul A. Heidenreich et al., 2011), the possibility of differences in plaque components and hemodynamic differences
remains to be studied. From a clinical perspective, regardless of differences in carotid artery
disease and the underlying etiology, both carotids are always included in imaging assessments.

1.4.3 Ischemic Stroke

Strokes are broadly classified as ischemic or hemorrhagic, reflecting the underlying etiology. Nearly 85% of strokes are ischemic, and 15% are hemorrhagic. Carotid atherosclerosis causes 20% to 30% of ischemic strokes (see Figure 1.17).

![Figure 1.17: Classification and Estimates of the Frequency of Stroke Etiology. Reproduced from (Albers, Easton, Sacco, & Teal, 1998) with permission (License: 3850340870541).]

The etiology of stroke includes extracranial vasculature atherosclerosis that affects the aortic arch or the vertebrobasilar arteries, intracranial penetrating artery atherosclerosis or disease, cardioembolism related to atrial fibrillation, or cardiogenic emboli or valve disease (see Figure 1.18).
The relationship of the carotid with ischemic stroke was first documented in ancient Greece. "Carotid," from the Greek word “karos,” means “to stupefy.” Compression of the carotid arteries was known to induce stupefaction, as evidenced by art found in the architecture of the Parthenon (Munster, Thapar, & Davies, 2016). The art on the column of the Parthenon shows a centaur compressing the neck of a Lapith during the mythological battle of Centauromachy. In 400 B.C., Hippocrates was among the first people to propose that “unaccustomed attacks of numbness and anesthesia” may predict apoplexy (Robicsek, Roush, Cook, & Reames, 2004).

Reports between 1600 and the 1900s presented several advances concerning the link between the carotids and stroke. Wepfer observed a disrupted blood supply at the carotid in four autopsy cases with apoplexy (1658) (Munster et al., 2016). Van Swieten proposed the theory that cerebral embolism causes strokes (1754), although he treated no cases of this condition (Munster et al., 2016). Carswell found that the brain softened with carotid occlusion or carotid division.
occlusion (Carswell, 1838). Kussmaul, a student of Virchow, reported that carotid artery thrombosis was associated with ipsilateral blindness (Kussmaul, 1872).

The most definitive link between carotid stenosis and ischemic stroke was reported in the mid-1900s. Moniz presented the first carotid arteriogram in 1927 (Doby, 1992). Using carotid arteriograms in 1951 and 1954, Miller Fisher presented the first definitive observations concerning carotid stenosis and stroke (Fisher, 1951, 1954). In his 1951 paper, Fisher postulates that bypass and anastomosis of an occluded carotid would be feasible. Soon after, on August 7, 1953, DeBakey performed the first successful carotid endarterectomy, resulting in a 19-year-long event-free rate, at which point the patient died from coronary occlusion (DeBakey, 1975). In 1980, Charles Kerber performed the first angioplasty (Kerber, Cromwell, & Loehden, 1980). The reader is referred to a more comprehensive review of the history of carotid stroke (Munster et al., 2016) summarized above.

In the 1990s, trials investigated whether carotid endarterectomy based on the stenosis grade improved clinical outcomes. Well known trials include the North American Symptomatic Carotid Endarterectomy Trial (NASCET) (N. A. S. C. E. T. Collaborators, 1991), the European Carotid Surgery Trial (ECST) (E. C. S. T. C. Group, 1998), the Asymptomatic Carotid Atherosclerosis Study (ACAS) (E.C.A.C.A.S., 1995), and the Asymptomatic Carotid Surgery Trial (ACST) (M. A. C. S. T. A. C. Group, 2004). Clinical trials have demonstrated the benefit of carotid endarterectomy in moderate and severe grade carotid artery disease among symptomatic patients. Collectively, the recommendations from these trials include surgical intervention for moderate and severe symptomatic carotid stenosis and severe asymptomatic carotid stenosis in patients with less than 3% perioperative risk.

Among asymptomatic patients, however, the management of carotid artery disease remains highly controversial (Naylor, 2015). Optimal medical therapy is considered the standard of care, and carotid endarterectomy is typically only considered in these asymptomatic patients if they have greater than three to five years of life expectancy, less than 3% risk of perioperative death, and are younger than 70 years of age (Hussain, 2015). Based on the ACST, which included all patients with asymptomatic moderate carotid artery stenosis, a 43% relative risk reduction in the five-year risk of disabling stroke from 6.1% to 3.5% was observed for patients
on optimal medical therapy versus those on optimal medical therapy who had undergone carotid endarterectomy, respectively. Stroke rates in patients with moderate carotid artery disease remain high, and whether surgical intervention is warranted is unclear (Naylor, 2015). Although current care is based on the clinical evaluation of risk factors such as age, perioperative mortality, and life expectancy (Hussain, 2015), many of these patients will develop stroke. Stroke rates are as high as 16% at 10 years (Halliday, 2010). More precise tools such as vessel wall imaging for risk-stratification of carotid artery disease are required.

Improved techniques may also reveal complicated carotid atherosclerosis as a source of thromboembolism in cryptogenic stroke. Cryptogenic stroke is a major diagnostic dilemma, with as many as a quarter of stroke patients and half of transient ischemic attack (TIA) patients presenting with no underlying reason for stroke or TIA. Stroke and TIA patients, despite <50% ipsilateral carotid stenosis according to Trial of ORG 10172 in Acute Stroke Treatment (TOAST) (Adams et al., 1993) and less than 70-99% carotid stenosis according to ASCOD (atherosclerosis, small-vessel disease, cardiac pathology, other causes, dissection) (Amarenco et al., 2013), may be classified as cryptogenic, more recently termed embolic stroke of undetermined source (ESUS). Paroxysmal atrial fibrillation and complicated carotid atherosclerosis are thought to be likely underlying reasons for ESUS. As many as 16.1% (Gladstone et al., 2014) to 30% (Rizos et al., 2012) of patients with cryptogenic stroke or TIA have atrial fibrillation. These patients are often overlooked with routine monitoring techniques due to paroxysmal atrial fibrillation. In the EMBRACE trial (Gladstone et al., 2014), a prospective randomized control trial of a cohort of adults ≥55 years of age with cryptogenic stroke, patients assigned to a diagnostic strategy of prolonged 30-day event-triggered electrocardiography (ECG) monitoring (intervention) compared with conventional 24-hour ECG monitoring (control) had an improved diagnosis of atrial fibrillation lasting 30 seconds or longer within 90 days (16% vs. 3.2%).

Similarly, nonroutine evaluation of the carotid arteries using advanced imaging has suggested that even in patients with less than 50% stenosis, the carotids may be a source of thromboembolism in ESUS stroke. For example, carotid plaque MRI showed that complicated plaque components are often present in patients with ESUS and low-grade stenosis (Cheung et
al., 2011; Freilinger et al., 2012; Gupta et al., 2016; Gupta, Gialdini, et al., 2015). Carotid atherosclerosis is associated with ischemic stroke, and advanced imaging strategies may be particularly useful for the evaluation of cerebrovascular disease in patients with asymptomatic and ESUS.

1.4.4 Systemic Disease

Because atherosclerosis is a systemic disease, carotid atherosclerosis may indicate the presence of disease in other vessels, including the coronaries, ileofemoral arteries, and abdominal aorta (Gallino et al., 2014). Patients such as those with peripheral vascular disease are often comprehensively examined for the presence of disease in other vascular beds such as the carotids (Qureshi et al., 2007). The systemic nature of atherosclerosis presents the possibility of identifying coronary atherosclerosis with noncoronary disease characterization (Gallino et al., 2014). The identification of polyvascular disease may be significant because polyvascular or multifocal disease patients are at higher risk of adverse clinical outcomes, including cardiovascular death, nonfatal myocardial infarction (MI), nonfatal stroke, and hospitalization, and a greater number of outcomes are associated with a greater number of locations of symptomatic disease (Sirimarco et al., 2013; Steg et al., 2007). Steg et al. have reported that these cardiovascular outcomes affect 5.31% of patients with only risk factors. In contrast, in patients with one, two, and three symptomatic arterial locations, cardiovascular outcomes may occur in 12.58%, 21.14%, and 26.27% of patients, respectively ($P<0.001$ for trend) (Steg et al., 2007). Whether carotid plaque characterization can improve risk stratification or provide insights into high-risk patients remains to be tested and is one area that is investigated further in this thesis.

1.5 Carotid Artery Stenosis

Landmark carotid artery trials such as NASCET and ECST recognized stenosis as a major risk factor for stroke. Clinical practice necessitates the evaluation of stenosis because major trials studied surgical and medical interventions for patients with stenosis. The NASCET and ECST trials describe two methods that are commonly used to quantify carotid stenosis. NASCET effectively quantifies stenosis based on the luminal reduction compared with the internal carotid
artery (ICA) distal outflow (Allan J. Fox & Singh, 2015). The method described by the ESCT trial measures the luminal reduction of stenosis and compares this value to the unseen ICA bulb width visible on standard angiography (the stenotic atheroma covers the ICA bulb). This ECST percentage of stenosis is derived from a denominator of up to twice the diameter of the distal ICA, with the ICA bulb being the largest part of the artery.

![Figure 1.19: Measuring Stenosis Using the NASCET Method. The degree of stenosis = 1 - N/D. Reproduced from (A J Fox, 1993) with permission (Received Mar 10, 2016).](image)

The NASCET method for measuring carotid stenosis on angiography is employed most commonly in current practice settings. The degree of carotid stenosis, defined as percent carotid stenosis, is calculated using the formula 1 - N/D (see Figure 1.19). N indicates the numerator, which is measured at the narrowest portion of the stenosis. D indicates the denominator and is measured well beyond the tapering of the carotid bulb where the wall of the internal carotid artery becomes parallel.

Several pitfalls are possible when measuring NASCET stenosis in clinical practice and research. The use of the NASCET method for percent stenosis without complying with specific details of how measurements are obtained is known to occur (A. J. Fox et al., 2009). A stenosis that is characterized as moderate according to NASCET criteria may be identified as severe if it is not properly measured (Allan J. Fox & Singh, 2015).
The lack of assessment for near occlusion is another concern when measuring stenosis. The nearly complete collapse of the ICA lumen beyond a very severe stenosis (see Figure 1.20) is referred to as a near occlusion in the NASCET trial.

**Figure 1.20:** Near Occlusion in Carotid Artery Disease. There is a collapsed lumen beyond very severe stenosis with flow reduction associated with a presumed physiological loss in the diameter of the distal ICA. Reproduced figure published by Wolters Kluwer Health, Inc., from (N.A.S.C.E.T. Collaborators, 1991) (License 3833990759397).

Less well identified is a minor physiological loss of diameter in the ICA beyond severe stenosis that can be considered “normal-looking” but otherwise exhibits partial collapse (Bartlett, Walters, Symons, & Fox, 2006; A. J. Fox et al., 2005). Both categories are referred to as “near occlusion” in the NASCET (N.A.S.C.E.T. Collaborators, 1991) representing complete and partial collapse. These near occlusions cannot be correctly measured and calculated for percent stenosis because the distal ICA diameter is too small and deformed. Researchers in the NASCET trial did not obtain measurements for near occlusion because the yielded percent can be substantially lower than that in severe stenosis. Using a partially collapsed denominator for a percent calculation would artificially reduce the percent stenosis, with some cases of severe...
stenosis calculated as 60% or less. NASCET findings suggest that before the measurement of any stenosis, the carotid should first be evaluated for the presence of a subtle near occlusion.

The value obtained from the measurement of the distal ICA diameter, well beyond the bulb where the walls are parallel, was used as the denominator in the calculation of percent stenosis in the NASCET trial. This strategy was used because the shape of the ICA bulb transitions from wide to narrow. Measuring the ICA at positions where it is larger than the distal ICA (where the walls are parallel) falsely increases the calculated percent stenosis (A. J. Fox et al., 2009).

Stenosis can be measured using various modalities, including ultrasound, MRI, CTA, and invasive digital subtraction angiography (DSA). Ultrasound is the most commonly performed technique for screening carotid stenosis, whereas CTA is more useful in the acute stroke setting. Follow-up imaging of patients with known stenosis commonly consists of magnetic resonance angiography. Magnetic resonance angiography is less operator dependent, does not expose the patient to radiation, can be conducted without contrast injection, is noninvasive, and allows for luminal evaluation from the aortic arch to the intracranial vasculature.

DSA is considered the gold standard for accurate measurement of stenosis but may be reserved for patients undergoing intervention (see Figure 1.21).
Figure 1.21: Internal Carotid Artery Stenosis Diagnosed with Invasive Digital Subtraction Angiography. Invasive digital subtraction angiography is considered the gold standard for measuring carotid artery stenosis. Reproduced from (Hyde et al., 2004) with permission (License 3802180168941).

Rates of neurological complication associated with catheter cerebral angiography have been reported to be approximately 2.6%, with a 0.14% rate of permanent stroke (Kaufmann et al., 2007). Features such as ulcers, calcifications, and dissections can be visualized. False positives may result from washed-out contrast. Artifacts from anything dense in the region of the carotid may hinder the evaluation of stenosis. Catheter-based angiographic studies remain the most accurate means of assessing the degree of carotid stenosis and exhibit relatively low complication rates, with an extensive study showing deaths and permanent stroke in 12 and 27 of 19,826 patients, respectively (Kaufmann et al., 2007).

Doppler ultrasound is widely used for the classification of the stenosis grade. Compared with arteriography, Doppler ultrasound is excellent for the classification of the stenosis grade (Grant et al., 2000); however, it exhibits subpar performance for determining the exact degree of stenosis and limited utility for the evaluation of near occlusion. Peak systolic velocity or peak systolic velocity ratio in the common carotid artery relative to the internal carotid artery is
usually converted to estimate carotid stenosis. The wide standard deviations of velocity for any given stenosis grade (see Figure 1.22) demonstrate the limited use of Doppler ultrasound for determining the absolute stenosis value using velocity.

![Figure 1.22: Carotid Artery Peak Systolic Velocity on Ultrasound and its Corresponding Angiographic Stenosis. Ultrasound velocity in cm/sec for each decile of angiography-confirmed stenosis diameter in the internal carotid artery. Reproduced from (Grant et al., 2000) with permission (License: 02.10.2016).](image)

Standardization is required for vascular laboratories to determine corresponding cutoff points for velocity parameters used with true stenosis (see Table 1.3 for an example).

Ultrasound is operator dependent, and the measurement of stenosis requires knowledge of carotid anatomy to recognize kinking, contralateral disease, and the presence of plaque components such as calcifications. Ipsilateral flow ratios of the common carotid artery to the internal carotid artery may not be valid if a contralateral occlusion is present. The presence of dense calcifications may lead to inaccuracy, and the evaluation of tandem lesions may be difficult due to the limited field of view.
Table 1.3: Ultrasound Velocity Cut-off Points to Classify Grade of Carotid Stenosis. Reproduced from (Grant et al., 2000) with permission (License: 02.10.2016).

<table>
<thead>
<tr>
<th>Degree of Stenosis (%)</th>
<th>Primary Parameters</th>
<th>Additional Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICA PSV (cm/sec)</td>
<td>Plaque Estimate (%)</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;125</td>
<td>None</td>
</tr>
<tr>
<td>&lt;50</td>
<td>&lt;125</td>
<td>&lt;50</td>
</tr>
<tr>
<td>50–69</td>
<td>125–230</td>
<td>≥50</td>
</tr>
<tr>
<td>≥70 but less than near occlusion</td>
<td>&gt;230</td>
<td>≥50</td>
</tr>
<tr>
<td>Near occlusion</td>
<td>High, low, or undetectable</td>
<td>Visible</td>
</tr>
<tr>
<td>Total occlusion</td>
<td>Undetectable</td>
<td>Visible, no detectable lumen</td>
</tr>
</tbody>
</table>

CTA is a vital part of the evaluation of an acute stroke patient. The need for a rapid assessment of stroke patients is driven, in part, by the need to assess the proximal anterior circulation for thrombi that are treatable with intra-arterial therapy (Berkhemer et al., 2015). CTA of the carotid artery is limited due to radiation exposure and the use of iodinated contrast agents. The carotids are included as part of the CTA acquisition, allowing for the evaluation of carotid stenosis.

CTA permits visualization of the carotid arteries from the aortic arch to the intracranial collaterals. Multidetector computed tomography (MDCT) permits the acquisition of 1- to 2-mm slices with a single breath hold. Current multisection computed tomography (CT) scanners enable the acquisition of up to 16 sections for each gantry rotation. Each rotation may require as little as 0.4 seconds. Axial collimation for cervical-cerebral CTA is performed using a collimation of 0.75 mm with a reconstruction of 1.5 mm for each axial image.

Carotid stenosis measurement is more reliable with CTA compared with DSA (Silvennoinen, Ikonen, Soinne, Railo, & Valanne, 2007). Intracranial disease is also reliably assessed with CTA (Nguyen-Huynh et al., 2008). Axial images, multiplanar reformatted images, and 3-Dimensional or 3D volume maximum intensity projection (MIP) and volume-model images contribute to the sensitivity and accuracy of multisection CTA. The ability to review images in various planes and reformats mitigates potential issues posed by superimposing
structures such as veins. Features including ulceration and calcification are easily evaluated at the time of stenosis measurements (see Figure 1.23).

![Figure 1.23: Computed Tomography Angiography of Carotid Artery Disease Demonstrating Ulceration and Stenosis. Ulceration (dashed arrow), stenosis (solid arrow) and the external carotid artery (arrowhead) are seen. Reproduced with permission from (Bartlett et al., 2006) (License 501105841).]

CTA images are acquired rapidly, with no issues related to pacemakers or implants. The 3D reformats are available to delineate the anatomy and simultaneously evaluate the proximal carotid, arch, and intracranial vasculature. Some limitations to the measurement of stenosis include the presence of calcifications or high-density artifacts, artifacts related to motion, or poor cardiac function. Intravenous contrast requires the rapid injection of approximately 150 ml of 300 to 320 mg/ml of nonionic contrast agent. A density of at least 150 Hounsfield Units (HU) is typically required, with imaging initiated immediately before the contrast density peaks in the carotid artery (Nadalo, 2015).

Magnetic resonance angiography (MRA) is commonly used to evaluate carotid stenosis (see Figure 1.24). The MRA field of view (FOV) allows visualization of the carotid artery from the aortic arch to the circle of Willis. Post-processing of MRI images, such as the creation of
multiplanar reformats (MPRs), MIPs, and 3D reconstructions, may facilitate the diagnosis of stenosis (Runck, Steiner, Bautz, & Lell, 2008).

![Image of Time-of-flight Angiography Demonstrating Stenosis at the Origin of the Internal Carotid Artery](image)

**Figure 1.24:** Time-of-flight Angiography Demonstrating Stenosis at the Origin of the Internal Carotid Artery. Figure reproduced with permission from (Alan R. Moody & Singh, 2015) (License: 3857810000492).

Both time-of-flight (TOF)-MRA and contrast enhanced (CE)-MRA possess comparable sensitivity and specificity for detecting severe stenosis and total occlusions. TOF-MRA is slightly less sensitive for the evaluation of carotid stenosis compared with CE-MRA. A summary of data from a meta-analysis performed by Debrey et al. is shown in Table 1.4.
Table 1.4: Sensitivity and Specificity of Time-of-Flight and Contrast Enhanced Magnetic Resonance Angiography for Evaluation of Stenosis against Gold Standard Digital Subtraction Angiography. Rounded figures from (Debrey et al., 2008).

<table>
<thead>
<tr>
<th>Degree of Stenosis</th>
<th>TOF-MRA</th>
<th>CE-MRA</th>
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<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>100</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
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<td>88</td>
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<tr>
<td>50-70</td>
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<td>92</td>
</tr>
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</table>

More recent studies specifically investigating 3T have also found that 3D TOF-MRA and CE-MRA at 3T are reliable tools for detecting high-grade proximal ICA stenoses (70-99%) (Weber et al., 2015). This study has suggested that TOF-MRA may misclassify pseudo-occlusions as complete occlusions. Comparison of 2-dimensional (2D) TOF-MRA to CE MRA has also revealed that the addition of gadolinium does not offer an advantage for distinguishing surgical or high-grade stenosis (Babiarz et al., 2009). Some authors have suggested that TOF-generated reformats are more likely to agree with stenosis grading compared with CE-MRA (Runck et al., 2008). TOF-MRA is also highly concordant with CTA, to a greater extent than CE-MRA (Lell et al., 2007). Others, however, have proposed that CE-MRA provides better image quality, improves diagnostic confidence, and has better inter-observer agreement (Mitra et al., 2006). Overall, CE-MRA is mostly used as a primary MRI modality, especially for 3T field strength magnets, to prevent the overestimation of stenosis by TOF-MRA (Platzek, Sieron, Wiggermann, & Laniado, 2014), in the absence of contraindications for gadolinium or for more accurate characterization of lower grades of stenosis.

1.6 Carotid Intima-Media Thickness

The Atherosclerosis Risk in Communities (Chambless et al., 1997) and Rotterdam (van der Meer et al., 2004) studies have demonstrated the relationship of carotid intima-media thickness (cIMT) with cardiovascular outcomes, including myocardial infarction, stroke, and mortality. They suggest that cIMT may be useful for the reclassification of patients from intermediate risk to higher cardiovascular risk. Proposed clinical applications include the use of cIMT among intermediate-risk or hypertensive patients for primary prevention of cardiovascular events.
There is some debate on the true benefit of routine screening of patients with cIMT to assess the risk of initial coronary events. Despite some of the prospective data suggesting the use of cIMT for reclassification of cardiovascular risk, the American College of Cardiology (ACC)/American Heart Association (AHA) guidelines do not endorse the use of cIMT for the detection of a first event (Goff et al., 2014). The information provided by cIMT in terms of Framingham Risk Scores is limited. Data from 14 population-based cohorts, including data for 45,828 individuals, indicated that cIMT provides small but likely not clinically significant value to the Framingham Risk score (Den Ruijter, Peters, Anderson, & et al., 2012). Serial imaging for changes in cIMT are also not recommended (Touboul et al., 2012) because it does not predict cardiovascular outcomes (Lorenz et al., 2012). Concerns regarding cIMT measurement quality have contributed to the ACC/AHA recommending against routine screening for cIMT (Goff et al., 2014).

B-mode or 2D ultrasound allows evaluation of the intima-media thickness of the vessel wall. Ultrasound imaging of the intima-media thickness is perceived as simple, readily available, and unlikely to produce incidental findings. Image acquisition and measurement, however, are operator-dependent. Clear reporting of the type of IMT value (e.g., mean or max), wall used (e.g., near or far), carotid segment (e.g., CCA), software (e.g., automated or manual and type) and angle of the operator at the time of measurement (e.g., single or multiple) may be required to interpret images or to allow comparisons between serial measures. The standardization of carotid intima-media thickness protocols and interpretations in research and clinical medicine has been proposed (Stein et al., 2008). Standardized imaging of carotid intima-media thickness necessitates the following protocols from larger studies such as the Atherosclerosis Risk in Communities (ARIC) or Multi-Ethnic Study of Atherosclerosis (MESA) (see Figure 1.25) to be able to apply the cIMT findings of these studies to cardiovascular outcomes.
Figure 1.25: Common Carotid Intima-media Thickness as Measured in the MESA Trial. The progression of common carotid intima-media thickness was found to predict stroke. The rectangular box delineates the area in which measurements were obtained and is approximately 0.5 to 1 cm below the carotid bulb (indicated by the vertical line). The arrow is pointing to focal thickness before the carotid blood suggestive of early plaque formation and was excluded from the measurements. Figure reproduced with permission from (Polak, Pencina, O'Leary, & D'Agostino, 2011) (License 3839510217834).

According to epidemiological studies, the interpretation of normal cIMT values can be adjusted for data specific to age, gender, and race/ethnicity. Increasing age is associated with thickening of the intima-media. Typically, ultrasound screening in these studies defined a normal cIMT thickness of less than 0.9 mm at the far wall of the distal common carotid artery. Advanced carotid plaque imaging may provide further cardiovascular and cerebrovascular risk stratification information.

1.7 Advanced Carotid Plaque Imaging: Modalities, Biomarkers, and Cardiovascular Disease

Advanced carotid plaque imaging allows for improved visualization of the vessel wall compared with carotid stenosis and intima-media thickness imaging (see Figure 1.26). Plaque burden, composition, and activity can be evaluated rather than lumen or wall thickness alone. The
information gleaned from imaging characteristics on burden, composition, and activity in the carotids may improve cardiovascular risk stratification and disease prevention.

**Figure 1.26:** Advanced Carotid Plaque Imaging for the Comprehensive Evaluation of Atherosclerosis. Magnetic resonance imaging allows depiction of several atherosclerotic components, including the lipid core (asterisk). The signal hypointensity (asterisk) indicates the lipid core of an eccentric atherosclerotic plaque with luminal preservation on fat saturation T1-weighted imaging pre- (a), and post-contrast (b). Multicontrast images usually also include T2 fat saturation (c), time-of-flight (d), and magnetization prepared rapid acquisition with gradient-echo (e). Figure from (Singh, Moody, Roifman, Bluemke, & Zavodni, 2015) without any changes and in accordance with the Creative Commons License (see http://creativecommons.org/licenses/by/4.0/legalcode).

Advanced imaging provides a dearth of biomarkers, “characteristic[s] that [are] objectively measured and evaluated as indicator[s] of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Atkinson, 2001). For example, measurements such as wall thickness or lipid core volume are indicators of the processes taking place in an atherosclerotic vessel wall (see Figure 1.27).
For biomarkers to be useful for cardiovascular risk stratification, they must add incremental information to traditional cardiovascular risk factors. The biomarker results should also impact clinical management in a meaningful way (T. J. Wang, 2011). Biomarkers should be associated with outcomes, should improve discrimination and calibration, and accurately reclassify a large proportion of patients.

Compared with more traditional serum biomarkers, imaging biomarkers allow direct visualization of an individual’s vessel wall disease. The potential utility of serum biomarkers that reflect inflammation and proteolysis of a vulnerable plaque has been suggested. These include
hs-CRP, IL-6, MMP-9, MMP-2, TIMP-1 and TIMP-2. Serum biomarkers may be nonspecific for the evaluation of a local plaque and may reflect processes in the noncarotid circulation. Similarly, imaging may provide more information than cardiovascular risk factor scores for incident cardiovascular events (Vlachopoulos et al., 2015; Zavodni et al., 2014; Pen et al., 2013). Measures from various modalities, including ultrasound, CT, FDG-PET, (Fludeoxyglucose-positron emission tomography) and MRI, are available for the evaluation of carotid arteries.

1.7.1 Ultrasound

The 2D total plaque area (TPA) and 3D total plaque volume (TPV) are two of the more contemporary measures proposed (compared with the intima-media thickness) for the evaluation of cardiovascular risk. The evaluation of larger regions of the vessel wall, particularly for the assessment of volumes, is thought to provide more accurate insight into a patient’s carotid disease burden. A more comprehensive evaluation compared with the intima-media thickness is reflected in the definition of the total plaque area, the longitudinally measured sum of the cross-sectional areas of the plaques of both carotids in the common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) (Spence et al., 2002). Figure 1.28 shows an example of plaque regression measured by the total plaque area in a patient over three months (Spence & Hackam, 2010). Such changes would likely be overlooked when measuring intima-media thickness (IMT).
Figure 1.28: Total Plaque Area Regression Using 2-Dimensional Ultrasound.

This plaque had also become denser with regression of the soft plaque, and increased calcification was observed in just over three months. Reproduced from (Spence & Hackam, 2010) with permission from Wolters Kluwer Health, Inc. (License 3840900998176).

Indeed, TPA has been suggested to be superior to IMT for the prediction of myocardial infarction events (AUC 0.64 versus 0.61) in a meta-analysis of data from 11 population-based studies (Inaba, Chen, & Bergmann, 2012). Over 10 years, the rates of MI were lower among subjects with a negative result on TPA (4%) vs. cIMT (4.7%). TPV, however, has been demonstrated to be superior for evaluation of the progression and regression of disease compared with TPA and IMT. In a study of 349 patients, TPV progression over a one-year time frame predicted stroke, death, or TIA (Wannarong et al., 2013). TPA predicted these outcomes weakly, whereas IMT was found to have no relationship with cardiovascular outcomes. The limitations of 2D ultrasound include potential imaging variability and arbitrary angle imaging of a thin plane, resulting in an incorrect diagnosis (Fenster, Downey, & Cardinal, 2001). While 3D ultrasound attempts to address some of these issues, immediate optimal rendering may not be available or may require complex intervention by the user.

While ultrasound is widely available and commonly used, its dependency on operator technique, as well as the limited spatial and contrast resolutions available, make it less preferable for evaluation of carotid plaque biomarkers. For example, carotid stenosis in surgical lesions (Johnson, Wilkinson, Wattam, Venables, & Griffiths, 2000) and plaque components (Y.
Watanabe et al., 2008) have been shown to be less accurately characterized relative to magnetic resonance angiography. Compared with histology, color Doppler is less sensitive, specific, and accurate than MRI for the diagnosis of at-risk soft plaques (sensitivity, 75 vs. 96%; specificity, 63 vs. 93%; accuracy, 69% vs. 94%, respectively) (Y. Watanabe et al., 2008). MRI depicted soft plaques are defined as containing substantial lipid and/or IPH components, and ultrasound soft plaques are defined as mostly echolucent.

1.7.2 Computed Tomography

The composition of carotid atherosclerotic plaques can be determined using CT. For example, in a multidetector CTA study of eight TIA patients undergoing carotid endarterectomy (CEA), it was found that plaque components could be accurately detected (Wintermark et al., 2008). CTA interpretation of plaque components requires automated classification algorithm software that relies predominantly on tissue density (see Figure 1.29).
Figure 1.29: In-Vivo Computed Tomography Angiography Image of the Common Carotid Artery and Matching Ex-Vivo Microcomputed Tomography and Histologic Sections. Automated classification computer algorithm-derived overlay showing the lipid-rich necrotic core (yellow), calcification (blue), blood products (red), and remaining connective tissue (green). Computed tomography angiography overlay demonstrating a plaque with a large lipid core, small calcifications, and an ulceration, making it a VIa lesion according to the American Heart Association classification, in agreement with the histologic examination, the gold standard for noncalcified carotid wall components, similar to ex-vivo microcomputed tomography, the reference for carotid wall calcium (specimens were decalcified before histologic sectioning). Reproduced with permission from (Wintermark et al., 2008) (License: 3854911432902).

Features on CTA may also indicate high-risk plaque components such as IPH. In a retrospective study, Eisenmenger et al. suggested that the presence of the calcified rim sign (see Figure 1.30) is strongly indicative of a soft plaque containing an IPH (Eisenmenger et al., 2016).
Similarly, U-King-Im et al. and colleagues suggested that ulceration (see Figure 1.31) is predictive of the presence of IPH. The combined and incremental value of various features such as the rim sign, ulceration, and density require further study to determine their utility for predicting stroke or other cardiovascular events rather than IPH. Well-designed prospective studies would certainly be of interest; however, the use of CT over other modalities such as MRI remains questionable from a cost, value, and radiation safety perspective.

**Figure 1.30:** Positive Rim Sign and Carotid Intraplaque Hemorrhage. In the upper panel, the carotid computed tomography angiography demonstrates positive rim signs (arrows) in both carotid plaques. In the lower panel, magnetization prepared rapid acquisition with gradient-echo with bilateral carotid intraplaque hemorrhage (arrows) in the same patient. Intraplaque hemorrhage was defined by magnetization prepared rapid acquisition with gradient-echo-positive plaque using a signal threshold of two-fold signal intensity over the adjacent sternocleidomastoid muscle. Reproduced with permission from (Eisenmenger et al., 2016) (License: 3865990342864).
CT exhibits improved spatial resolution compared with ultrasound; however, it exposes the patient to ionizing radiation and iodinated contrast. MRI, although not yet widely available, has greater diagnostic value than CT. Symptomatic carotid plaques are better characterized on MRI compared with multidetector computed tomography angiography (MDCTA), although MDCTA offers higher specificity at the cost of lower sensitivity (Grimm et al., 2014). In a study of 22 stroke unit patients with symptomatic unilateral carotid disease, Type VI AHA (American Heart Association) plaques identified with MRI (using T1, T2, and TOF) were found to better predict symptomatic disease (sensitivity, specificity, positive predictive value, and negative predictive value = 80%) relative to ulceration on MDCTA (sensitivity, specificity, positive predictive value, and negative predictive value = 40, 95, 89, and 61%, respectively) (Grimm et al., 2014). The performance of 3T MRI for carotid imaging is likely underestimated in this particular study, which did not employ 3D imaging sequences (discussed below) or sequences designed to detect Type VI AHA plaque features such as IPH (A. R. Moody et al., 2003; Ota, Yarnykh, et al., 2010).
Figure 1.31: Simultaneous Presence of Carotid Intraplaque Hemorrhage and Ulcer. (A) Magnetic resonance imaging depicting intraplaque hemorrhage (white arrow) as a strong signal within the wall of the left carotid bulb of a recently symptomatic patient. Sagittal (B), axial source image (C), and coronal computed tomography angiography reformat of the left carotid artery in the same patient revealing a large atherosclerotic plaque, causing only 50% stenosis but with a small ulcer (black arrow) within the plaque. Figure reproduced with permission from (U-King-Im et al., 2010) (License 3865970608471).

1.7.3 Positron Emission Tomography

Positron Emission Tomography (PET) is a nuclear medicine functional imaging technique that detects regional positron emission (gamma rays) from radiotracers, typically the glucose
analogue fludeoxyglucose (18-F). Uptake of 18-F increases proportionally with the metabolic activity that occurs during, e.g., inflammation or tumor growth. Carotid PET imaging has been shown to reveal plaque inflammation that may be associated with plaque instability and high-risk features of carotid plaques (see Figure 1.32) (Hyafil et al., 2016). However, any suggested incremental value of PET-MRI over MRI or PET-CT over MRI remains to be determined.

**Figure 1.32**: High-risk Carotid Plaque Characterized with Positron Emission Tomography Magnetic Resonance Imaging. Representative example of a high-risk carotid atherosclerotic plaque formed of lipid rich-necrotic core and intraplaque hemorrhage imaged with combined 18 F-FDG positron emission tomography and magnetic resonance imaging. Corresponding axial views of a
nonstenotic carotid atherosclerotic plaque acquired simultaneously using a combined imaging system including magnetic resonance imaging with successive high-resolution black-blood T2W (a), black-blood T1W before (b) and 5 min after injection of gadolinium chelates (c) and time-of-flight sequences (d) and positron emission tomography images (e) acquired 150 min after injection of 18 F-FDG, allowing perfect matching of both acquisitions (f; fusion image formed of time-of-flight and 18 F-FDG positron emission tomography images). Note the presence of a nonstenotic atherosclerotic plaque ipsilateral to the vascular territory of the stroke (a–d; white arrowheads) formed of a hypointense area on T1W, and T2W and time-of-flight sequences and a hyperintense area on T1W, T2W and time-of-flight sequences consistent with the presence of lipid rich-necrotic core associated with intraplaque hemorrhage type II. High accumulation of 18 F-FDG was detected with positron emission tomography in the corresponding area (e–f; white arrowheads), supporting the presence of elevated inflammatory activity in the atherosclerotic plaque. Reproduced with permission from (Hyafil et al., 2016) (License: 3866010395175).

1.7.4 Magnetic Resonance Imaging

Carotid MRI offers several advantages for evaluation of carotid disease biomarkers over other modalities. The modality permits accurate characterization and quantification of carotid biomarkers, does not require administration of iodine or ionizing radiation, and is not operator-dependent. Despite perceived limitations regarding availability, carotid MRI has been suggested to be cost-effective among select subgroups (Gupta, Mushlin, Kamel, Navi, & Pandya, 2015; Saam et al., 2013), clinically useful for identifying patients at risk of stroke (Gupta et al., 2013), and appropriate for identifying patients with a high-risk cardiovascular phenotype or risk of cardiovascular events (Noguchi, Yamada, Higashi, Goto, & Naito, 2011; Singh, Moody, Rochon-Terry, Kiss, & Zavodni, 2013; Sun et al., 2015; Zavodni et al., 2014). Carotid pulse sequences allow image acquisition within minutes (N. Balu et al., 2011).

Another advantage of carotid MRI is that it may already be included or appended to neurovascular imaging examinations to provide simultaneous information on cerebrovascular ischemic injury. For example, a 75-year-old male referred for peripheral vascular disease and
known carotid artery disease underwent neurovascular MRI in 2010 and 2012. A new white matter lesion was found in 2012 (see Figure 1.33), suggesting interim carotid plaque instability.

![Image of MRI scans](image)

**Figure 1.33:** Progression of White-Matter Disease. In these images, fluid-attenuated inversion recovery magnetic resonance imaging depicts a new white matter lesion (arrowhead) on the left in 2012 compared to 2010. Development of new white-matter lesion in a patient with ipsilateral carotid plaque containing ulcer and intraplaque hemorrhage. © Navneet Singh and Alan Moody, 2016. All rights reserved.

Time-of-flight magnetic resonance angiography shows the development of a new ulcer in the internal carotid artery, implicating the carotid artery as the source of the thromboembolism (see Figure 1.34).
Figure 1.34: Development of Carotid Artery Ulceration. Time-of-flight 3-dimensional magnetic resonance angiography demonstrates the development of a new ulcer (arrowhead) in a patient with progression of ipsilateral white-matter disease and underlying carotid intraplaque hemorrhage. Sagittal oblique reformats (a, d) and corresponding axial images at the level of the blue lines (b-c, e-f) are shown. © Navneet Singh and Alan Moody, 2016. All rights reserved.

The competitive edge of MRI lies in its ability to accurately characterize vessel wall plaque biomarkers, including components, plaque burden, and luminal stenosis. MRI can measure the wall thickness and include the adventitia, which is excluded on ultrasound (Zhang, Guallar, Qiao, & Wasserman, 2014). This adventitia may be especially important given that vasa vasorum initially proliferates in this region and is implicated in plaque progression related to IPH (Virmani, Kolodgie, & Burke, 2005). Initial carotid MRI investigations have validated 2D carotid imaging techniques against histology for plaque characterization using a combination of bright and dark blood acquisitions. Multicontrast imaging techniques employ multiple T1 and T2 weighting, as well as an option for the addition of gadolinium, allowing for assessment of disease burden, components, and activity. For example, standard carotid MRI acquisitions would include T1, T2, PD, T1 post-contrast, and time-of-flight angiography. Plaque components may be determined based on their signal characteristics in multicontrast magnetic resonance imaging. The high lipid (i.e., cholesterol and cholesterol ester) content of the necrotic core results in rapid
T2 decay and manifests as a hypointense signal on T2 images. Post-contrast imaging allows visualization of the fibrous cap and lipid core (Cai et al., 2005; Kwee et al., 2009). The lipid-rich necrotic core is visualized due to poor vascularity in post-contrast T1-weighted studies. IPH is identified by its T1 signal intensity and occurs as a result of the shortening of T1 caused by blood methemoglobin (see Figure 1.35).

![Figure 1.35: Magnetic Resonance Imaging of Carotid Vessel Wall and Intraplaque Hemorrhage.](image)

The temporal resolution of IPH imaging is on the order of minutes, and therefore, sequences for the evaluation of this plaque component can be appended to neurovascular imaging exams. In the previous example of the patient with the simultaneous presence of a
carotid ulcer and a new white matter lesion on follow-up imaging, the ulcer occurs at the site of prior IPH (see Figure 1.36) depicted on a black blood carotid MRI sequence.

Figure 1.36: Intraplaque Hemorrhage Underlying Patient with New Carotid Ulcer and White-Matter Lesion. Magnetic resonance-intraplaque hemorrhage (MR-IPH) 3-dimensional imaging (matrix, 272 x 224; field of view, 270 x 190 mm; slice thickness, 0.5 mm; 16-channel neurovascular coil) revealing intraplaque hemorrhage (arrowhead) at the site of subsequent internal carotid artery ulceration in 2012. A large amount of intraplaque hemorrhage underlies the ulceration site in 2010 (a, c), with residual intraplaque hemorrhage remaining in 2012 (outlined by the yellow-dashed line) (e, f). Sagittal oblique reformats (a, d) and corresponding axial images at the level of the blue lines (b-c, e-f) are shown. © Navneet Singh and Alan Moody, 2016. All rights reserved.

Various histological correlation studies have demonstrated the ability of MRI to accurately and directly visualize the lipid-rich necrotic core, fibrous cap, and IPH (Bitar et al., 2008b; Cai et al., 2005; Cappendijk et al., 2008; A. R. Moody et al., 2003; Yuan et al., 2001). Table 1.5 summarizes the features of plaque components on carotid MRI based on various histology-imaging correlation studies.
Table 1.5: Plaque Component Signal Characteristics on Carotid Magnetic Resonance Imaging. Table from (N. Singh et al., 2015) without any changes and in accordance with the Creative Commons License (http://creativecommons.org/licenses/by/4.0/legalcode).

Typical 2D multicontrast MRI permits the acquisition of images encompassing 3 to 4 cm of the carotid artery (Yuan et al., 2001). The protocols are centered at the carotid bifurcation (Yuan et al., 2001), a region with a predilection for atherosclerosis, and result in 2-mm-thick slices in the through-plane direction. Thus, 3 or 4 cm of coverage would result in 16 to 20 slices. However, earlier studies appeared to only match four to six locations across multiple acquisitions to the histological findings (Yuan et al., 2001). Most of these sequences also employed a 256 x 256 matrix with a 13-cm field of view. This would result in a voxel size of 0.5 (in plane x) by 0.5 (in plane y) by 2 mm (through plane z). To reduce voxel sizes and minimize partial volume artifacts, these protocols also employed a zero-filled Fourier transform, resulting in voxels of 0.254 by 0.254 by 2.0 mm (Yuan et al., 2001). To obtain black blood images for T1, T2, and proton density (PD) weightings, flow was suppressed via double inversion recovery. The 2D protocols also used fast spin echo. Furthermore, these protocols employed a custom-designed phased-array surface coil, 1.5T scanners, and cardiac gating. Early prospective trials established multicontrast MRI protocols (T. Saam et al., 2005; Yuan et al., 2001) and demonstrated the value of these protocols for the prediction of ischemic neurovascular events (Takaya et al., 2006).

Multicontrast MRI has various limitations, including a long acquisition time, small volumes of coverage, and variable image quality. Multicontrast imaging requires registration of multispectral acquisitions because the acquisitions are obtained separately for each contrast (e.g., T1, T2) at different times throughout the imaging protocols. Patient motion may lead to misregistration. The field of view is also limited to a few centimeters. Carotid MRI is, however, advancing from 2D to 3D imaging techniques. The use of 3D imaging will allow acquisitions from the Circle of Willis to the aortic arch, rather than bifurcation alone (A. R. Moody et al., 2003). As a result, long segments of the vessel wall can be evaluated more completely.
Furthermore, such 3D sequences can be acquired in four to five minutes, which may be particularly important for evaluating disease along the carotid artery, and in particular, some areas such as the aortic arch may be of interest in the work-up of stroke patients or for intervention planning. The aortic arch is often screened for disease prior to aortic clamping in the coronary artery bypass graft.

Hardware such as a surface coil may introduce signal heterogeneity (see Figure 1.37) (N. Singh et al., 2015). The use of neurovascular phased-array coils may be one solution to ameliorate this issue.

![Figure 1.37: Magnetic Resonance Imaging Surface Coils.](A) Dedicated Surface Coils Provide an Improved Signal-to-noise Ratio for Superficial Structures (A). When these coils are applied to a cylindrical water phantom measuring 6 cm in diameter, the drop-off in signal intensity on the T1-weighted images provides a visual demonstration of the penetration depth of the coil (B). Figure from (N. Singh et al., 2015) without any changes and in accordance with the Creative Commons License (http://creativecommons.org/licenses/by/4.0/legalcode).

Despite the promising nature of 3D carotid MRI for evaluation of carotid atherosclerosis, there are some challenges that would benefit from investigation. Flow suppression in 2D MRI usually employs flow suppression techniques such as double inversion recovery of motion sensitization. However, given that 3D volumes are on the order of 30 cm rather than 3 to 4 cm, flow suppression may be challenging. Motion-sensitized dephasing, for example, by motion-sensitized driven equilibrium (MSDE) preparation (J. Wang et al., 2007) requires that an image be acquired immediately after application and before recovery of the longitudinal magnetization vector (N. Balu et al., 2011). The length of the scan time using this strategy is four to five
promising sequences in 3D carotid MRI are emerging that may allow the simultaneous assessment of stenosis and the vessel wall in a single acquisition capable of imaging both bright blood and dark blood (J. Wang et al., 2013).

In addition to a shift from 2D to 3D imaging for evaluation of carotid plaque, there have also been improvements in the MRI field strength. Initial imaging was performed at 1.5 Tesla or 1.5T; however, 3T allows for greater signal-to-noise ratio (SNR). For example, when considering 3D magnetization-prepared rapid acquisition with gradient-echo (MPRAGE), image quality was very slightly improved but mostly retained at 3T compared with 1.5T (S. McNally et al., 2014) (mean 3.87 vs. 4.34 on a 5-point scale, P<0.0001). The reliability on 3T and 1.5T was similar and approximately 0.8 for inter-rater reliability and 0.9 for intra-rater reliability. The greater field strength may pose an issue for quantification of plaque components, such as calcium or IPH, which are more susceptible. Using an automated quantification algorithm, Kerwin demonstrated no significant differences in signal between 3T and 1.5T for plaque components, including IPH (Kerwin et al., 2008). The next section addresses advanced carotid MRI for cardiovascular disease prevention applications.

### 1.8 Carotid Magnetic Resonance-depicted Intraplaque Hemorrhage Imaging

Carotid MRI has the potential to provide information regarding various plaque components and morphological biomarkers. Among the various vessel wall plaque component biomarkers available through carotid MRI, IPH can be easily imaged, routinely investigated in the clinical setting, and provides useful clinical information. The presence of carotid IPH provides insight into cardiovascular risk and aids in identification of culprit carotid lesions in stroke patients. Carotid IPH has a hazard ratio of five to six for stroke, which outweighs any of the risk factors for stroke (Bonati & Nederkoorn, 2015). There is increasing interest in evaluation of this biomarker, given that the pathophysiology of IPH may enhance the progression of atherosclerosis, exhibit relationships with other cardiovascular outcomes such as myocardial infarction, and can be easily detected. The clinical feasibility and usefulness for identifying a
high-risk cardiovascular phenotype has prompted extensive MRI studies to evaluate IPH using 2D methods such as multicontrast imaging. Imaging of IPH using 3D modalities (see Figure 1.38) may be useful for routine clinical practice.

![Image of Three-Dimensional Carotid Magnetic Resonance Imaging of Intraplaque Hemorrhage. © Navneet Singh and Alan Moody, 2016. All rights reserved.](image)

**Figure 1.38:** Three-Dimensional Carotid Magnetic Resonance Imaging of Intraplaque Hemorrhage. © Navneet Singh and Alan Moody, 2016. All rights reserved.

The sequences required to apply this technique are readily available on all scanner platforms, images are rapidly acquired, IPH detection is accurate, and interpretation is simple, presently consisting of dichotomization of the results into the presence or absence of IPH. Furthermore, MRI is currently the only readily available imaging technique for the detection of plaque hemorrhage, and it is already used for investigations of carotid artery disease, thus making the addition of this one sequence to the standard carotid artery imaging protocol simple.
and causing little time-cost impact. Our institution has employed this 3D carotid IPH imaging approach (see Figure 1.38) as part of routine neurovascular imaging over the past decade. The experience provides an opportunity to better understand both the determinants and consequences of this biomarker. The thesis uses this IPH experience to address IPH and its age-specific sex differences in low-grade stenosis, role in ipsilateral embolic stroke of undetermined source, and the relationship with a high-risk cardiovascular phenotype. This work raises questions about the significance of quantification of IPH and its relationship with clinical outcomes. The final project in this thesis therefore demonstrates the reliability of a quantitative imaging analysis protocol that may be used future trials.

1.8.1 Carotid Artery Disease Evaluation Paradigm Shift and Knowledge Gaps

500,000 new stroke events occur each year in the United States (J. Broderick et al., 1998). Greater than 80% of strokes are ischemic, of which 15 to 20% result from carotid artery atherosclerosis. Carotid artery atherosclerosis management has been directed by evidence from NASCET and ECST; landmark carotid trials though dating from nearly 30 years ago (N.A.S.C.E.T. Collaborators, 1991; E. C. S. T. Group, 1998). In these trials, carotid atherosclerosis was estimated by measuring luminal stenosis as a surrogate. Patients found to have high-grade carotid artery stenosis presumably due to significant atherosclerotic disease were demonstrated to benefit from carotid endarterectomy. However, the number needed to undergo surgery to prevent a single stroke required as many as 8 to 83 patients, depending on the degree of stenosis and presence of symptoms (Gorelick, 1999). Patients with lower grades of stenosis however may have significant carotid plaque with the lumen maintained until later stages of disease in the progression of atherosclerosis. This phenomenon described by Glagov (Glagov et al., 1987), may represent a missed opportunity for stroke intervention. Direct visualization of carotid atherosclerosis disease burden and plaque components allows for identification of a high-risk group of patients at risk of end-organ events and opportunity to intervene. Magnetic resonance imaging may allow for the identification of plaque components at increased risk of causing clinical end-organ outcomes.
1.8.1.1 MR-IPH and Outcomes

Systematic reviews and meta-analyses have demonstrated that MRI identified plaque components such as intraplaque hemorrhage, fibrous cap thickness, and lipid core are associated with cerebrovascular events (Hosseini, Kandiyil, Macsweeney, Altaf, & Auer, 2013). Patients with IPH rather than other plaque components have higher odds of future cerebrovascular events (Hellings et al., 2010; Hosseini et al., 2013). Metanalyses including those by Saam and colleagues (Saam et al., 2013) and Gupta and colleagues (Gupta et al., 2013) have demonstrated a 5.7- and 4.6-fold higher risk of cerebrovascular events in patients with IPH. IPH also appears to perform better when compared to existing risk scores suggesting that direct visualization of plaque components may provide an opportunity for personalized risk stratification. Altaf and colleagues found that carotid IPH was associated with future cerebrovascular events whereas the ECST risk scores were not (Altaf et al., 2014). Prospective multicenter trials accumulating hundreds of patients with serial carotid MRI such as the Canadian Atherosclerosis Imaging Network Project 1 are anticipated to confirm these observations in the Canadian context (Tardif et al., 2013).

1.8.1.2 Gaps Overview

Understanding the relationship with risk factors, clinical outcomes, and natural history of IPH may be facilitated by an IPH imaging database. The paucity of carotid MRI in routine clinical practice is one hindrance to collecting a sizeable database of IPH imaging for research purposes. Accumulating a database of patients with carotid MRI including patients of low-grade stenosis is one particularly promising opportunity for studying intraplaque hemorrhage. A database of IPH imaging including low-grade stenosis patients would supplement studies seeking to understand the natural history and determinants of IPH (Kandiyil, Altaf, Hosseini, MacSweeney, & Auer, 2012), such as effect of age and sex, though limited to higher grades of stenosis. The database would also allow for study of stroke etiology in patients with embolic stroke of undetermined source (that by definition are deemed to have <50% stenosis). Knowledge gaps on carotid IPH and cardiovascular events could also be studied if the database were to be populated with data on cardiovascular history, imaging and clinical outcomes, and demographic information such as age,
sex and ethnicity. Others have demonstrated relationships of carotid intima-media thickness and cardiovascular events in large population studies (Polak et al., 2011), though few studies address the potential prognostic value of plaque components such as IPH.

In addition to testing hypotheses surrounding presence of IPH for the identification and risk stratification of a high-risk cardiovascular and cerebrovascular phenotype, whether IPH volume, location, age, surface features, co-plaque components, and geometry may improve reclassification and patient outcomes come into question. IPH depicting pulse sequences, image analysis tools and segmentation algorithms that are accurate, reliable and easy to use may assist in evaluating IPH more robustly. Furthermore, the use of the IPH imaging information gleaned either independently or in combination with patient’s status of circulating inflammatory markers, genetics, comorbidities, sex and ethnicity, requires further study for identification of high-risk patient groups and impact of these factors on IPH and outcomes. For example, genetics of a patient such as haptoglobin genotype may assist in the targeted imaging detection of IPH (Marvasti et al., 2018). Ultimately one goal is to target these high cardiovascular and cerebrovascular risk groups for prevention and intervention. Reliable quantification of IPH may be useful in evaluating response to therapy, testing for new therapeutic targets, and effectiveness of preventative efforts. In particular, methodologies with good inter-, intra-, and scan-rescan reliability of 3D carotid artery 3T MRI to volumetrically quantify IPH are among the next steps towards furthering the clinical translation of IPH imaging.

1.8.1.3 Potential IPH Imaging Impact

Carotid IPH may allow for detection of patients at risk of stroke that would benefit from CEA. Several trials have demonstrated the benefit of CEA in moderate and severe grade carotid artery disease among symptomatic patients, though even this subgroup may benefit from further risk stratification. Among asymptomatic patients, the management of carotid artery disease remains highly controversial (Naylor, 2015) and is one specific group in whom further risk stratification based on presence of IPH may be useful. OMT is considered the standard of care and CEA is typically only considered in these asymptomatic patients only if they have greater than 3 to 5 years of life expectancy, less than 3% perioperative death, and are younger than 70 years of age.
(Anas Hussain, Verma, Gupta, & Al-Omran, 2015). In asymptomatic moderate carotid artery disease, stroke rates are as high as 16% at 10 years (Halliday et al., 2010) and better tools for risk-stratification are required. Employing IPH imaging in specific groups such as asymptomatic moderate carotid artery disease patient group may potentially translate into saved lives through more precise identification of patients at high risk of stroke. A direct trial comparing incorporation of IPH into the management of carotid artery disease algorithm is clearly needed. To this end, various efforts, beyond the scope of this thesis incorporating such questions are currently underway including the ECST-2 (European Carotid Surgery Trial-2) and ACTRIS (Endarterectomy Combined with Optimal Medical Therapy (OMT) vs OMT Alone in Patients with Asymptomatic Severe Atherosclerotic Carotid Artery Stenosis at Higher-than-average Risk of Ipsilateral Stroke).
1.9 Aims and Hypotheses

We hypothesized that the presence of carotid MRI depicted IPH is associated with cardiovascular risk factors and consequences that indicate a high-risk cardiovascular and cerebrovascular phenotype (see Figure 1.39).

**H$_1$:** Presence of carotid MRI depicted IPH is associated with cardiovascular risk factors and consequences that indicate a high-risk cardiovascular and cerebrovascular phenotype

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**Figure 1.39:** Thesis Overview.

1.9.1 Age-Specific Sex Differences in MRI Depicted Carotid Intraplaque Hemorrhage

The aim of this project is to investigate age-specific sex differences in carotid MR-IPH between males and females. We hypothesize carotid MR-IPH is more prevalent in the carotid arteries of males compared to females, and age-specific sex differences in carotid MR-IPH converge between males and females with increasing age.
1.9.2 Carotid Magnetic Resonance Depicted Intraplaque Hemorrhage in Embolic Stroke of Undetermined Source

The aim of this project is to investigate whether carotid MR-IPH is present in embolic stroke of undetermined source patients. We hypothesize that carotid IPH depicted by MRI is associated with ipsilateral stroke or TIA among embolic stroke of undetermined source patients.

1.9.3 Identifying a High-risk Cardiovascular Phenotype by Carotid MRI-depicted Intraplaque Hemorrhage

The aim of this project is to determine the ability of MR-IPH to identify past cardiovascular events defined as MI, coronary intervention, and peripheral vascular disease (PVD) in patients investigated with MRI for neurovascular disease. We hypothesize patients investigated for neurovascular disease who are found to have MR-IPH have an increased number of prior cardiovascular events indicative of a high risk cardiovascular phenotype.

1.9.4 A Combined 3D Black-blood and Time-of-flight Magnetic Resonance Imaging Approach for the Volumetric Quantification of Carotid Artery Vessel Wall and Intraplaque Hemorrhage

The aim of this project is to investigate the inter-, intra-, and scan-rescan reliability of 3D carotid artery 3T MRI, using a 3D T1w gradient echo black blood (GRE BB) and TOF acquisition, to volumetrically quantify vessel wall and IPH and assess agreement with 2D T1 MRI for measuring vessel wall volume. We hypothesize that volumetric quantification of vessel wall (VW) and IPH is highly reliable on 3D carotid artery 3T MRI and that quantification of VW has excellent agreement with the 2D carotid artery MRI.
Chapter 2—Age-Specific Sex Differences in Magnetic Resonance Imaging Depicted Carotid Intraplaque Hemorrhage

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2.1 Overview

**Background and Purpose:** Stroke rates are higher in males compared to females in the fourth through seventh decades of life, and higher rates may result from differences in carotid intraplaque hemorrhage (IPH), an unstable atherosclerotic plaque component. We report age-specific sex differences in the presence of MRI depicted carotid IPH. **Methods:** Patients (n=1115) underwent MRI for carotid IPH between 2005 and 2014. Low-grade carotid stenosis patients (n=906) without prior endarterectomy were eligible for this cross-sectional study. **Results:** Of the 906 patients included (mean age ± SD in years, 66.98 +/- 15.15), 63 (6.95%) had carotid IPH. In males and females, carotid IPH was present in 11.43% (48/420) and 3.09% (15/486), respectively (p<0.0001). Multivariable logistic regression analysis confirmed greater odds of carotid IPH in males for all ages: 45 to 54 (OR=45.45, 95% CI 3.43 to 500), 55 to 64 years (OR=21.74, 95% CI 3.21 to 142.86), 65 to 74 years (OR=10.42, 95% CI 2.91 to 37.04), and ≥75 years (OR=5.00, 95% CI 2.31 to 10.75). Male sex modified the effect of age on the presence of carotid IPH (β= 0.074, SE=0.036, p=0.0411). **Conclusions:** Males have greater age-specific odds of MRI depicted carotid IPH compared to females. With increasing age post-menopause, the odds of carotid IPH in females becomes closer to that of males. Delayed onset of carotid IPH in females, an unstable plaque component, may partly explain differential stroke rates between sexes and further studies are warranted.

2.2 Introduction

Stroke incidence is lower in females compared to males between the ages of 45 to 74 years (Kissela et al., 2004; Reeves et al., 2008). The difference in stroke incidence, however, is eliminated after 75 years of age (Kissela et al., 2004; Reeves et al., 2008). The protection from stroke conferred upon females between the ages of 45 to 74 years may be related to the protective effects of pre-menopausal sex hormones that delay the onset of atherosclerosis (Jousilahti, Vartiainen, Tuomilehto, & Puska, 1999; Yahagi, Davis, Arbustini, & Virmani, 2015). Women have been reported to have less plaque burden and more stenosis compared to men (Iemolo, Martiniuk, Steinman, & Spence, 2004), suggesting that sex hormones play a role in
arterial remodeling. Data on the age-specific sex differences in carotid plaque components and their natural course is sparse.

Pathology and imaging studies have reported carotid atherosclerosis composition differences between the sexes (Ota, Reeves, et al., 2010; Ota et al., 2013). One specific plaque component, intraplaque hemorrhage (IPH), is a biomarker of plaque instability associated with the progression of atherosclerosis (Takaya et al., 2005), a high-risk cardiovascular phenotype (Noguchi et al., 2011; Singh et al., 2013), and future cerebrovascular outcomes including stroke (Gupta et al., 2013; Saam et al., 2013; Singh et al., 2009; Singh, Zavodni, & Moody, 2012). Several recent studies point to IPH as a potential source of thromboembolism among patients with embolic stroke of undetermined source, which by definition have non-stenotic carotid artery disease (<50%) (Freilinger et al., 2012; Gupta et al., 2016). Studies are needed understand the determinants of IPH in low-grade (<50%) carotid stenosis and data on the age-specific sex differences in carotid plaque components and their natural course is sparse in these patients. Further, prior studies have demonstrated that male sex and age are associated with the presence of IPH (Ota, Reeves, et al., 2010; Ota et al., 2013; van den Bouwhuijsen et al., 2012). However, whether sex modifies the effect of age remains to be investigated.

MRI can identify carotid IPH (Bitar et al., 2008; A. R. Moody et al., 2003; N. Singh et al., 2015; Yuan & Parker, 2016). Validated carotid IPH pulse sequences such as 3D T1-weighted gradient echo (GRE) (A. R. Moody, Allder, Lennox, Gladman, & Fentem, 1999; A. R. Moody et al., 2003; A. R. Moody & Singh, 2016) may be implemented into routine clinical protocols (A. R. Moody & Singh, 2016). 3D T1-weighted GRE is routinely combined with magnetic resonance angiography (MRA) at our institution providing an opportunity to study the IPH biomarker in a large clinical population.

The objective of this study was to report age-specific sex differences in MRI depicted carotid IPH in an institutional sample comprising ten years of data. Given that age-specific stroke rates are lower in females between age 45 to 74 compared to males, carotid artery disease is a major cause of stroke, and females may have greater proportions of stable plaque components, we hypothesized that age-specific sex differences in carotid IPH exist to help explain previously reported differences in stroke rates. Specifically, we hypothesized that males
have greater age-specific odds of MRI depicted carotid IPH compared to females and that with increasing age, the odds of carotid IPH in females becomes closer to that of males. We report age-specific sex differences in the presence of MRI depicted carotid IPH in patients with less than 50% stenosis.

2.3 Materials and Methods

Institutional ethics board approval was received for this retrospective study and the requirement for informed consent was waived.

2.3.1 Participants and Study Groups

Patients (n=1115) referred for neurovascular MRI undergoing 3D T1-weighted GRE black-blood imaging for the presence of carotid IPH between 2005 and 2014 were considered for study inclusion. Study exclusion criteria included i) carotid artery disease ≥50% stenosis to exclude patients with higher plaque volumes given that plaque volume and stenosis are positively correlated(Rozie et al., 2009), and ii) patients with prior carotid endarterectomy since plaque components may have been altered as a result of iatrogenic causes.

Age groups were defined *a priori* as < 45 years, 45 to 54 years, 55 to 64 years, 65 to 74 years, and ≥75 years paralleling studies of age-specific sex differences in stroke such as The Greater Cincinnati/Northern Kentucky Stroke Study (Broderick et al., 1998; Kissela et al., 2004). Patients undergoing neurovascular MRI including 3D T1 weighted GRE imaging at our institution in this study included suspected neurovascular disease patients (usually symptomatic) referred by physicians, including stroke neurologists for neurovascular evaluation.

2.3.2 Carotid MRI Protocols

3D T1-weighted GRE imaging allows for the identification of carotid IPH (Bitar et al., 2008; A. R. Moody et al., 2003). This acquisition exploits the T1-shortening effects of methemoglobin resulting in high signal intensity that indicates the presence of carotid IPH (See Figure 2.1 and Figure 2.2) (Leung & Moody, 2010).

3D T1-weighted GRE imaging was performed on a Philips Medical System Scanner with a 16 channel neurovascular coil employing a T1-weighted fat-saturated (using a selected water excitation RF pulse) fast field echo sequence, with imaging acquired in the coronal plane.
(repetition time (TR), 11 msec; echo time (TE), 4 msec; field of view (FOV), 270x190 mm²; matrix, 512x256 mm²; slices, 100; through-plane thickness, 0.5mm; voxel size 0.5 mm x 0.7 mm x 0.5 mm interpolated. Imaging was also performed on a GE 1.5 Tesla MR (Twinspeed) with an eight-channel neurovascular phased-array coil (USA Instruments, Aurora, Ohio) using a T1-weighted fat saturated (Special [spectral inversion at lipids]; GE Healthcare) GRE sequence (TR, 6.7 msec; TE, 1.7 msec; flip angle, 15°; field of view, 300 mm²; matrix size, 320 × 320; through-plane thickness, 2 mm; pixel size, 0.94mm × 0.94mm × 1 mm). 3D T1-weighted GRE imaging is robust, vendor agnostic, and similar IPH characteristics have been demonstrated at both 3T and 1.5T field strengths (S. McNally et al., 2014).

**Figure 2.1:** MRI Depicted Carotid Intraplaque Hemorrhage Indicated by Signal Hyperintensity in the Vessel Wall. *Singh et al. Stroke.* 2017. 48(8): 2129-2135.
2.3.3 Carotid IPH Imaging Analysis and Reliability

Carotid IPH is defined as plaque signal intensity that exceeds the intensity of the adjacent sternocleidomastoid by 50% (Altaf et al., 2014; Hosseini, Kandiyil, Macsweeney, Altaf, & Auer, 2013; Mendes, Parker, Kim, & Treiman, 2013; Murphy et al., 2003; Singh et al., 2013). Since patients at our institution underwent imaging with neurovascular rather than surface coils, issues of coil sensitivity related to magnetic field inhomogeneity were not as relevant as in surface coil dependent imaging. Experienced neuroradiologists (range of experience, 15 to 40 years) and a cardiothoracic radiologist with subspecialty and academic expertise in vascular biology imaging (A.R.M., experience, 27 years), routinely report the presence of IPH on the clinically acquired 3D T1-weighted GRE imaging acquisition. Increased signal intensity was considered to be carotid IPH if it was in the carotid artery wall. The dichotomous interpretation of carotid IPH has well established reliability (Ota, Yarnykh, et al., 2010; S. McNally et al., 2014) and kappa coefficients are 0.75 and 0.9 for inter- and intra-observer reliability (A. R. Moody et al., 2003). Concordance of clinical reports and an independent rater (N.S.) (n=50) for carotid IPH was found to have a kappa of 0.902 (95% CI, 0.769 to 1.000, SE 0.068).
2.3.4 Clinical Records Review

Data collection included abstraction of data from neurovascular MRI reports and patients’ charts using available physician consultation letters. Patients’ electronic and paper charts were reviewed for cardiovascular risk factors, medication history, and atherosclerotic end-organ events including history of transient ischemic stroke, stroke, and myocardial infarction. Traditional cardiovascular risk factors were included since they are independently associated with cardiovascular outcomes and therefore confound the evaluation of the effect of age and sex on the presence of carotid IPH.

2.3.5 Source Data Collection, Organization, and Storage

Between 2003 and 2014, charts of a total of 2,102 patients (n=4204 carotid arteries) undergoing neuro-vascular MRI including carotid imaging with 3D T1 weighted GRE acquisition at our academic tertiary care center underwent review. Patients undergoing neurovascular MRI including 3D T1 weighted GRE imaging at our institution were categorized into two subgroups based on referral source: i) patients with established peripheral vascular disease, typically referred by vascular surgeons for screening (usually asymptomatic) of the patients’ carotid arteries as suggested by vascular guidelines (Qureshi et al., 2007), and ii) suspected neurovascular disease patients (usually symptomatic), referred by physicians including stroke neurologists for neurovascular evaluation. The imaging database provides unique data on IPH imaging since the 3D T1 weighted GRE was included as part of routine clinical care at our academic tertiary care center. The opportunity to ascertain IPH imaging was undertaken in part due to the relative paucity of information available on carotid IPH, an emerging carotid biomarker, compared to other biomarkers such as carotid intima-media thickness. For example, GoogleScholar and PubMed return 116,000 and 9,318 results, respectively, for “carotid intima-media thickness,” compared to only 7,120 and 371 for “carotid intraplaque hemorrhage” (search date, July 2016). The sequence necessary for acquisition of this plaque component is not typically considered a routine clinical sequence since the current standard of carotid imaging for clinical decision-making is based on the lumen, or carotid stenosis. Therefore, the goal of data collection for this database was to identify patients with and without the presence of MRI
depicted carotid IPH, as well as ascertain available information regarding demographic data, cardiovascular risk factors, cardiovascular imaging test results, and clinical cardiovascular outcomes. Such a database would allow for study of carotid IPH associated risk factors and consequences. Data collection was structured to abstract data from various aspects of the patients' charts, including neurovascular MRI reports, cardiovascular imaging reports such including Doppler reports, and clinical medical history from the patients’ charts using available physicians' consultation letters. Figure 2.3 overviews the components of data collection used in this 10-year institutional experience of magnetic resonance depicted IPH imaging.

![Data Collection Overview](image)

**Figure 2.3:** Data Collection Overview.

Each patient’s clinical records were searched for information on cardiovascular risk factors, medical history, medication history, carotid imaging, and atherosclerotic end-organ events. Charts were abstracted for presence of vascular risk factors, including hypertension, diabetes, hypocholesterolemia, atrial fibrillation, smoking status, and family history of coronary artery disease. These traditional cardiovascular risk factors were included since they are well known to be independently associated with future clinical cardiovascular outcomes and are
therefore included in cardiovascular risk-factor studies. Multimorbidity or the concomitant presence of multiple risk factors is also associated with multiplicative mortality risk (The Emerging Risk Factors Collaboration, 2015). Relevant vascular medical histories, including past coronary artery disease, myocardial infarction, coronary interventions (angioplasty, stenting, or coronary artery bypass graft), stroke, transient ischemic attack, amaurosis fugax, carotid endarterectomy, carotid dissection, carotid aneurysm, intracerebral hemorrhage, aortic aneurysm, patent foramen ovale, and fibromuscular dysplasia, were collected. This past medical history is routinely recorded data in charts indicating a patient’s coronary or cerebrovascular status (e.g., prior stroke and myocardial infarction). Features such as patent foramen ovale, atrial fibrillation, and proximal vessel wall dissections attempted to capture noncarotid artery atherosclerosis causes of stroke. Intracerebral hemorrhage was collected to allow for differentiation of ischemic versus nonischemic stroke. Information on causes of stroke as well as stroke type provides a potential avenue to explore the brain and carotid IPH link.

Medication history using a created drug reference allowed for documentation of medication use. Patients’ medication use for the following categories was recorded: hypertension (angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, calcium-channel blockers, beta-blockers, and diuretics), diabetes (insulin, metformin, biguanides, glitazones, and sulfonylureas), and nitroglycerin. These medications were selected to comprehensively collect cardiovascular medications. Some of these medications have also been implicated in the pathophysiology of IPH, although the data have been mostly cross-sectional and therefore have only suggested associations rather than establishing cause and effect. As more insights are gained into the pathophysiology of IPH, these medications become important to consider while evaluating the relationship of carotid IPH and other cardiovascular risk factors and outcomes. While attempts were made to obtain information that was as granular as possible at the time of chart review, ultimately, data had to be collapsed as is discussed in the "Data Cleaning, Validation and Handling of Missing Data" section due to the limitations of this dataset.

Carotid MRI data points from neurovascular imaging reports included stenosis grade or stenosis grade category and presence of IPH. Stenosis grade and IPH status are routinely reported for neurovascular MRI at our institution by subspecialty academic radiologists with
knowledge and interest in carotid artery imaging. In patients with multiple neurovascular MRI examinations, the results of three consecutive MRI reports were recorded. Any available ultrasound and computed tomography angiography (CTA) reports were also abstracted for stenosis grade category. The presence of carotid ulceration was recorded based on CTA reports, given that prior studies suggest ulceration to be a high-risk feature associated with the presence of MR-IPH (U-King-Im et al., 2010). The data variables collected allowed for determination of stenosis grade progression for patients who had serial imaging, change in IPH status, and time-to-event or time-to-follow-up information. Time-to-follow-up data were calculated as the number of days between two serial imaging scans. Table 2.1 summarizes the data variables collected.

Data were entered directly into spreadsheets. In some cases, electronic case report forms and paper forms were converted to Excel format and were used to prepare the data for cleaning and validation steps. Data storage was also achieved using spreadsheets. Patients identified were kept separately to comply with Research Ethics Board approval, ensure privacy of the patients included in the database, and follow good research practice guidelines (Breault, 2013). Unique identifiers were kept for each record in the database. Each record in the database was stored in a row. Standardized reorganization of data was carried out. Text was coded numerically where possible with “0” indicating "No" or "Absent" and “1” indicating "Yes" or "Present." Similarly, ordinal categorical variables were ordered numerically, with larger numbers indicating a higher rank order. Standardized reorganization for data to numerical form, when possible (e.g., conversion of clinical stenosis grade from 0 to 3 representing <50%, 50 to 70%, 70 to 99%, and 100% stenosis), facilitated data analysis. Database collection was achieved with the assistance of supervised chart abstracters. Training included didactic teaching on the order of several hours per abstracter and case-by-case review of queries throughout the data abstraction. Queries were typically resolved on the day made or as close to the day made as possible, and chart abstraction was periodically reviewed to ensure accuracy of data collection. Patients were randomly selected for review of their entered data throughout the data collection process.
<table>
<thead>
<tr>
<th>Table 2.1: Summary of Data Variables Collected.</th>
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</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
</tr>
<tr>
<td>ID</td>
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<tr>
<td>Date of Birth</td>
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<tr>
<td>Sex</td>
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<tr>
<td><strong>Vascular Risk Factors</strong></td>
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<td>Hypertension</td>
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<td>Diabetes Mellitus</td>
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<td>Dyslipidemia</td>
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<td>Atrial Fibrillation</td>
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<tr>
<td>Smoker, Any</td>
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<tr>
<td>Smoker, Present</td>
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<tr>
<td>Family History of Coronary Artery Disease</td>
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<tr>
<td><strong>Relevant Medical History</strong></td>
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<td>Coronary Artery Disease</td>
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<td>Coronary Artery Bypass Graft</td>
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<tr>
<td>Coronary Angioplasty or Stenting</td>
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<tr>
<td><strong>Cerebrovascular</strong></td>
</tr>
<tr>
<td>Stroke</td>
</tr>
<tr>
<td>Transient Ischemic Attack</td>
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<tr>
<td>Amarosis Fugax</td>
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<tr>
<td>Carotid Endarterectomy</td>
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<tr>
<td>Carotid Dissection</td>
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<tr>
<td>Carotid Aneurysm</td>
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<tr>
<td>Intracerebral Hemorrhage</td>
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<tr>
<td><strong>Other Vascular</strong></td>
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<td>Abdominal Aortic Aneurysm</td>
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<td>Patent Foramen Ovale</td>
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<tr>
<td>Fibromuscular Dysplasia</td>
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<tr>
<td><strong>Medications</strong></td>
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<tr>
<td>Hypertension</td>
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<td>Angiotensin Converting Enzyme Inhibitor</td>
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<td>Calcium Channel Blocker</td>
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<tr>
<td>Beta-blocker</td>
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<tr>
<td>Diuretic</td>
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<tr>
<td>Angiotensin II Receptor Blockers</td>
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<td>Statin</td>
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<td>Antiplatelet</td>
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<tr>
<td>Anticoagulant</td>
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<tr>
<td>Nitroglycerin</td>
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<tr>
<td><strong>Diabetes, Any</strong></td>
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<tr>
<td>Insulin</td>
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<tr>
<td>Metformin</td>
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<tr>
<td>Biguanide</td>
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<tr>
<td>Glitazone</td>
</tr>
<tr>
<td>Sulfourea</td>
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<tr>
<td><strong>Cardiovascular Outcomes</strong></td>
</tr>
<tr>
<td>First Clinical Event Subsequent to First MRI</td>
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<tr>
<td>Date of First Clinical Event Subsequent to First MRI</td>
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<td>Second Clinical Event Subsequent to First MRI</td>
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<td>Date of Second Clinical Event Subsequent to First MRI</td>
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<td>Third Clinical Event Subsequent to First MRI</td>
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<td>Date of Third Clinical Event Subsequent to First MRI</td>
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<td>Fourth Clinical Event Subsequent to First MRI</td>
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<td>Date of Fourth Clinical Event Subsequent to First MRI</td>
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<tr>
<td>Death</td>
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<tr>
<td><strong>Imaging</strong></td>
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<tr>
<td>First MRI</td>
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<tr>
<td>Date of First MRI</td>
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<tr>
<td>Presence of Right Carotid IPH</td>
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<tr>
<td>Presence of Left Carotid IPH</td>
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<tr>
<td>Degree of Right Carotid Stenosis</td>
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<tr>
<td>Degree of Left Carotid Stenosis</td>
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<tr>
<td>Second MRI</td>
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<tr>
<td>Date of Second MRI</td>
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<tr>
<td>Presence of Right Carotid IPH</td>
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<tr>
<td>Presence of Left Carotid IPH</td>
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<tr>
<td>Degree of Right Carotid Stenosis</td>
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<tr>
<td>Degree of Left Carotid Stenosis</td>
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<tr>
<td>CTA</td>
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<tr>
<td>Date of First CTA</td>
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<tr>
<td>Degree of Right Carotid Stenosis</td>
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<tr>
<td>Degree of Left Carotid Stenosis</td>
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<tr>
<td>Degree of Right Carotid Stenosis</td>
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<tr>
<td>Degree of Left Carotid Stenosis</td>
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<tr>
<td>Presence of Uler</td>
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<tr>
<td>Doppler Ultrasound</td>
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<tr>
<td>Date of Doppler Ultrasound</td>
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<tr>
<td>Degree of Right Carotid Stenosis</td>
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<tr>
<td>Degree of Left Carotid Stenosis</td>
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</tbody>
</table>
2.3.6 Data Cleaning, Handling of Missing Data, and Validation

The collected database underwent cleaning and validation steps to ensure that the data used were internally consistent or reliable and produced accurate results. The approach to data cleaning included: i) identifying invalid data, ii) investigating reasons for any invalid data, iii) reviewing charts to address any invalid data, iv) spot checking random patient charts to ensure accuracy of data, and v) testing validity, both intra-rater, inter-rater, and clinical report concordance for carotid IPH status and clinical stenosis grade, the key imaging parameters of interest in this body of work. Both manual and semi-automated steps were used to address each data cleaning and validation step.

In the first step, manual inspection of the data was carried out to identify any obvious issues and ensure the face validity of the database. Irregular or nonsense entries were corrected or removed. This step included addressing visible issues for each case such as removing text from a date field or converting text into numbers. Responses for each variable were numerically standardized, including conversion of text stating a stenosis range to a categorical response. Dates were formatted to ensure uniformity using an eight-character standardized classification system. It was ensured that serial imaging was entered in chronological order with the earliest scans being recorded as the first imaging tests and the latest scan being recorded as the last imaging tests. No cases or variables had to be dropped from the database based on the face validity step. During evaluating face validity, it was apparent that several variables had low response rates, and therefore a strategy employing the exclusion of these variables was used.

Code was written using SAS® (version 9.4) to provide for additional data validation and cleaning steps of the dataset. Data variables were relabeled to ensure clarity during data cleaning and analysis steps. Automated checks included searches for outliers (range check), format (character check), and proper documentation of response categories (category checks). Specifically, validation for continuous variables included searches for outliers through range searches and determining the frequency of missing data. Variables with less than 10 data points were dropped from the database given that meaningful analysis would likely not be possible. Furthermore, if only less than 10 responses were recorded, it was assumed that the data on the variable were not regularly abstracted from charts, and these variables were deleted from the
database entirely. Otherwise, incomplete data points variables with greater than 10 responses were identified as missing data points and coded in the database as “.” Ten responses were selected as an arbitrary cut-off point representing a threshold of 0.5% of the potential available dataset. The threshold was set at this level to ensure that potential subset analyses on rarer conditions would be possible. For example, the threshold also allowed us to capture data of patients with rare conditions such as fibromuscular dysplasia of the neurovasculature, which may affect as few as 0.3 to 3% of patients (Varennes et al., 2015). While the data were retained in the database, this did not necessarily translate into inclusion in the data analyses of individual projects. The data variables would need to be considered on a project by project basis. The decision to retain information is for potential review of more interesting cases, exploratory analysis, and case-control type studies.

Records that could not be verified, such as those with missing subject identification numbers without corresponding hospital file numbers in the link-list file, were discarded from the database. Furthermore, duplicate entries were screened and reconciled. The detection of duplicate entries was automated, and reconciliation was manual. In most instances, one of the duplicate entries identified only had a subject identifier without any clinical data, so the nonmeaningful record was removed, leaving the meaningful entry in the database. Reconciliation of the records in which duplicate cases had meaningful clinical data was done via manual review of the record and consolidation of information into one record. A limited number of cases with discordant information prompted chart review of those cases. Case-by-case review for face validity of data and random data checks were undertaken throughout the data collection and cleaning processes. Table 2.2 provides the database cleaning steps.
**Table 2.2**: Database Cleaning Steps.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Records</th>
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<tbody>
<tr>
<td>• Relabeling of data variable names</td>
<td>• Verification of records</td>
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<tr>
<td>• Visualization for obvious problems (face validity)</td>
<td>• Reconciliation of duplicate data</td>
</tr>
<tr>
<td>• Correction of date formats</td>
<td>• Case by case review (face validity)</td>
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<tr>
<td>• Conversion of text into numerical responses</td>
<td>• Random checks of record</td>
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<tr>
<td>• Standardization of numerical responses</td>
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<tr>
<td>• Validation of continuous variables</td>
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<tr>
<td>• Validation of categorical variables</td>
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<tr>
<td>• Handling of variables with insufficient data</td>
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<tr>
<td>• Setting of missing value codes</td>
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<tr>
<td>• Handling of missing data for retained variables</td>
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<tr>
<td>• Verification of records</td>
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<td>• Reconciliation of duplicate data</td>
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<tr>
<td>• Case by case review (face validity)</td>
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<tr>
<td>• Random checks of record</td>
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Various strategies may be used in the statistical handling of missing data in large databases, including collapsing data. In our case, data were collapsed when necessary to maximize its usability and minimize missing data at the cost of making it more difficult to answer more granular questions. To maximize usable data on carotid stenosis, degree of carotid stenosis was collapsed into four groups, <50%, 50 to 70%, 70 to 99%, and 100% or total occlusion. Data on medications were also collapsed. For example, charts were reviewed for specific medications such as type of antihypertensive (beta-blockers, calcium-channel blockers, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs)). Since the specific type of antihypertensive was not always available, considering presence or absence of antihypertensive medication in analyses allowed for retention of more records in the database.

Of the 2,102 patients with 3D T1 GRE imaging between 2005 and 2014, 1,115 comprised the source database. Patients were removed from the database if they did not have a
neurovascular MRI report (n=21), observations were not linkable to the master list and therefore no corresponding hospital file number was available to verify any data (defined as a nonverifiable record) (n=93), or their carotids may have been investigated because of known peripheral vascular disease rather than for suspected neurovascular disease (n=661). The reason for excluding patients with peripheral vascular disease was to ascertain a more homogenous group of low-grade stenosis patients with as low as possible a burden of atherosclerosis to allow for improving internal validity.

2.3.7 Statistical Analysis

Continuous variables with a normal distribution were expressed as a mean and accompanied by a standard deviation. Comparisons of age between males and females were made using an unpaired two-sided t-test. Pooled rather than Satterthwaite p-values were reported since equal variances could be assumed. Categorical variables were reported as counts and percentages, and were compared between the sexes using a two-sided Chi-squared test. Comparisons of binary matched data for differences in carotid IPH in the right versus left carotid arteries were made using a two-sided McNemar’s test.

Logistic regression was used to evaluate the relationship of sex and carotid IPH. Multivariable logistic regression covariates included hypertension, dyslipidemia, diabetes mellitus type two, and smoking history. To evaluate age-specific sex differences in carotid IPH, we included an age-by-sex covariate interaction term in reference and adjusted models. Statistical analysis was performed using SAS Version 9.4 (SAS Institute, Cary, NC, USA). A p-value of less than 0.05 indicated a statistically significant difference.

2.4 Results

Nine hundred and six patients (mean age ± SD in years, 66.98 +/- 15.15) were eligible for study (See Figure 2.4).
Carotid IPH was found in 63 (6.95%) of included patients. Demographic, clinical characteristics and carotid IPH presence are summarized in Table 2.3. Males had greater proportions of hypertension, dyslipidemia, type 2 diabetes, and smoking history.
In males and females, carotid IPH was present in 11.43% (48/420) and 3.09% (15/486), respectively (p<0.0001). Figure 2.5 depicts the proportion of patients with carotid IPH stratified by sex and age. No patients had carotid IPH under the age of 45. Between ages 45 to 54 years, only two males had carotid IPH. Females less than the age of 65 were not found to have carotid IPH. Table 2.4 includes the proportions of males and females with carotid IPH by age group.
Based on logistic regression, compared to women, men were found to have greater odds of carotid IPH at ages 45 to 54 (OR=52.63, 95% CI 4.27 to 1000), 55 to 64 (OR=25.00, 95% CI 3.88 to 166.67), 65 to 74 (OR=11.90, 95% CI 3.39 to 41.67) and ≥ 75 years (OR=5.56, 95% CI 2.61 to 11.76).

Multivariable logistic regression confirmed that males have greater odds of carotid IPH than females at ages 45 to 54 (OR=45.45, 95% CI 3.43 to 500), 55 to 64 (OR=21.74, 95% CI 3.21 to 142.86), 65 to 74 (OR=10.42, 95% CI 2.91 to 37.04) and ≥ 75 years (OR=5.00, 95% CI 2.31 to 10.75).

The odds of carotid IPH was greater with age in both males and females, and the effect of carotid IPH on age was modified by sex in both univariable (β=0.075, SE=0.0352, p=0.0312) and multivariable (β= 0.0737, SE=0.0361, p=0.0411) logistic regression. The odds of IPH among females approached that of males with age as shown in the unadjusted and adjusted odds of age-specific sex differences in carotid IPH reported in Table 2.4.
Bilateral carotid IPH was found in nine of 420 (2.14%) males, and two of 486 (0.41%) females and significantly differed between the sexes (p=0.0287). Males in the older age categories had higher proportions of bilateral IPH (see Table 2.4).

Of the 52 patients with only unilateral carotid IPH, 32 (61.54%) and 20 (38.46%) patients had an affected left and right side, respectively (p<0.0001). In these patients with unilateral disease, the left side was affected in 53.85% (7/13) of females (p=0.0006) and 64.10% (25/39) males (p<0.0001).

Table 2.4: Age-Specific Differences in Carotid Intraplaque Hemorrhage between Men and Women.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Carotid IPH Presence, %</th>
<th>Reference Model*</th>
<th>Adjusted Model†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any</td>
<td>Unilateral Only</td>
<td>Bilateral Only</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>&lt;45</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>(0/30)</td>
<td>(0/60)</td>
<td>(0/30)</td>
</tr>
<tr>
<td>45 to 54</td>
<td>3.33</td>
<td>0.00</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>(2/60)</td>
<td>(0/63)</td>
<td>(1/60)</td>
</tr>
<tr>
<td>55 to 64</td>
<td>11.34</td>
<td>0.00</td>
<td>9.28</td>
</tr>
<tr>
<td></td>
<td>(11/97)</td>
<td>(0/95)</td>
<td>(9/97)</td>
</tr>
<tr>
<td>65 to 74</td>
<td>11.11</td>
<td>2.04</td>
<td>9.26</td>
</tr>
<tr>
<td>≥75</td>
<td>18.40</td>
<td>7.65</td>
<td>15.20</td>
</tr>
</tbody>
</table>

* The reference model shows the odds of carotid intraplaque hemorrhage in males versus females, and was adjusted for sex and a sex-by-age interaction term.
† The adjusted model shows the odds of carotid intraplaque hemorrhage in males versus females and was adjusted by hypertension, dyslipidemia, smoking history, diabetes, sex, and sex-by-age interaction term.
2.5 Discussion

2.5.1 Findings
The data in this single institution study revealed that in patients with low-grade carotid artery stenosis, carotid IPH occurs after the age of 45 years, is present in less than 1% of patients before the age of 55 years, and by the age of 75 years, affects as many as 12% of all patients. Females were not affected by carotid IPH before the age of 65 years. Moreover, males have greater odds of carotid IPH compared to females, and with increasing age post-menopause, the odds of carotid IPH in females also increases and becomes closer to that of males. In other words, sex modifies the effect of age on carotid IPH. To our knowledge, no other studies have investigated or demonstrated the convergence of risk of carotid IPH between the sexes with increasing age. The infrequency of carotid IPH, an unstable plaque component associated with future stroke, in women before the age of 75 may in part explain the reason that women are relatively protected from stroke compared to men before the seventh decade of life. Our findings additionally suggest a delayed onset of carotid IPH in females.

2.5.2 Novelty
This study comprising nearly ten years of data, as a result of institutional incorporation of 3D T1-weighted carotid MRI into routine neurovascular imaging, provided an opportunity to test our hypothesis that carotid IPH exhibits age-specific sex differences. Thus, an opportunity was afforded to evaluate the age-specific sex differences in a large group of patients without significant carotid artery stenosis or higher pre-test probabilities of vessel wall disease. The use of a large homogenous group of low vessel wall volume disease patients reduces confounding factors in evaluating the relationship of age and sex that might be associated with higher-grade disease such as lifestyle differences. Since only a few minutes additional scan time is necessary to evaluate for IPH within existing neurovascular MRI protocols, it was possible to capture a relatively large dataset of patients with low-grade carotid stenosis with information on their IPH status.
2.5.3 Other Studies
Observational and population studies including MRI of plaque components have found that age and sex are determinants of carotid IPH (Ota et al., 2013; Kandiyil et al., 2012; Cheung et al., 2011). Overall, our data found carotid IPH in 11% and 3% of males and females, respectively. Ota and colleagues found a prevalence of 24% and 6% (Ota et al., 2013). The higher prevalence of carotid IPH in the latter study may be attributed to Ota using an overall higher-risk group, low-grade carotid stenosis arteries of patients with contralateral moderate-to-high grade carotid stenosis. Similarly, Kandiyil investigated patients with moderate and severe carotid stenosis; in their sample and meta-analysis they found that women with symptomatic carotid stenosis were less likely to have IPH than men (Kandiyil, Altaf, Hosseini, MacSweeney, & Auer, 2012). In our study, the carotid IPH status in patients with bilateral low-grade stenosis was captured by incorporating carotid IPH imaging into routine clinical neurovascular MRI. This is in contrast to other studies and trials that select patients for vessel wall imaging as a result of their carotid stenosis (Ota et al., 2013) or intima-media thickness.

In our study, men had 4.1 (95% CI, 2.2 to 7.4) greater odds of carotid IPH compared to women, which is similar to some reports (Ota et al., 2013). While our confidence intervals overlap, our point estimate is higher compared to a population-based study reporting that plaques from males had a 2.2 (95% CI, 1.7 to 2.9) greater odds of IPH compared to females (van den Bouwhuijsen et al., 2012). One reason for the difference in our study may be that, among the patients referred for neurovascular MRI and selected for <50% stenosis, rather than known carotid artery disease, a greater proportion of females may have been referred for MRI for neurovascular symptoms ultimately unrelated to carotid artery disease.

Men and women also had a slightly greater and statistically significant prevalence of carotid IPH within the left carotid artery. Ischemic stroke is more often diagnosed in the left hemisphere (Hedna et al., 2013), and the underlying etiology of this discrepancy is unclear. This predilection may in theory be due to differences in shear stress resulting from anatomical differences between the left and right carotid arteries and plaque components. While the increased diagnosis of stroke in the left hemisphere may be related to the underdiagnoses of right hemisphere stroke in clinical practice (Paul A. Heidenreich et al., 2011; Portegies et al., 2015),
the possibility of differences in plaque components and hemodynamic differences remains to be rigorously studied. Recently, differences in plaque components have been described and our findings of sidedness are in line with these (Selwaness, van den Bouwhuijsen, van Onkelen, et al., 2014). Together, the findings of sidedness and lack of symmetry in carotid IPH between the carotid arteries suggest that both local and systemic factors play a role in the development of carotid IPH.

2.5.4 Strengths
The strengths of the present study included the power to ascertain age-specific differences in carotid IPH with MRI using data acquired in the routine clinical setting that allowed us to capture a large dataset of patients with bilateral low-grade carotid stenosis. The benefit of extracting our study sample from a clinical setting allows both potential application of any results to the clinical population of the sample studied (i.e., external validity), as well as the ability to apply strict inclusion and exclusion criteria to ensure sufficient homogeneity allowing for greater internal validity. In addition to being rapid, the 3D T1-weighted GRE acquisition used is scanner agnostic and multi-vendor for the diagnosis of carotid IPH. The acquisition can also be incorporated into routine clinical protocols (A. R. Moody & Singh, 2016) as demonstrated in the group within this study. We chose to use this particular pulse sequence since it does not require surface coils and has been validated using neurovascular coils that are routinely available in the clinical setting (A. R. Moody et al., 2003). The IPH biomarker is also detectable with other validated MRI acquisitions.

2.5.5 Limitations
This study had several limitations. First, the study was cross-sectional and retrospective rather than longitudinal and prospective. Serial imaging studies are required to further understand the natural history of carotid atherosclerosis and provide deeper insights into the natural course of IPH. Such trials are underway, and their results are anticipated (Tardif et al., 2013). Serial imaging studies involving advanced imaging are costly, and this study exploited an opportunity to collect a large set of data in the course of routine clinical practice. While understanding the incidence of disease would require cohort studies, cross-sectional studies cost-effectively provide
information on prevalence of disease. Second, information on age at menopause and menarche, parity, and use of hormone replacement therapy, were not consistently available from charts. In developed countries, the average age of natural menopause of 51 years is used as a reference in cardiovascular studies (Appiah et al., 2016), and we interpreted our data with this knowledge. Third, while we were able to restrict our analysis to patients with less than 50% stenosis to exclude patients with higher plaque volumes given the correlation of stenosis with plaque volume (Rozie et al., 2009), our analyses were not adjusted for degree of stenosis. Finally, we did not evaluate plaque components such as lipid core, fibrous cap or calcium, and any age-specific sex differences they may exhibit. Carotid IPH is emerging as a critically important plaque component in prediction of end-organ events. Time constraints limit routine inclusion of multi-contrast acquisitions necessary to identify other plaque components in routine clinical practice, though more recent technological advancements in pulse sequence design may address this issue in the future.

2.5.6 Future Studies
Differences in sex-steroids may explain the observations in this study including the lower odds of carotid IPH in women per decade of life, the delayed onset of IPH in women, and convergent risk between men and women with increasing age. Estrogen is known to play a role in the age-specific sex differences in cardiovascular disease and the 10 to 15 year delayed onset of coronary disease in women (Jousilahti et al., 1999; Yahagi et al., 2015). Histopathology studies of the coronaries have demonstrated that post-menopausal women are at an increased risk of plaque rupture (Burke, Farb, Malcom, & Virmani, 2001). Carotid endarterectomy specimen studies have shown that plaques obtained from women are less inflammatory with lower macrophage and interleukin-8 content, and with greater smooth muscle and collagen (Hellings et al., 2007). Plaque stabilization may be achieved via anti-inflammatory effects of estrogen modulated by its antioxidant and anti-apoptosis effects (Nofer, 2012). Furthermore, estrogen may confer protection against atherosclerosis via several mechanisms including the promotion of endothelial mediated dilation and blood flow, and cerebrovascular reactivity. Nitric oxide synthase and nitric oxide generation are increased by estrogen and may explain in part the protective role of estrogen in atherosclerosis (Nofer, 2012). While the effects of estrogen on IPH require further
investigation, estrogen’s ability to attenuate the inflammatory processes that have been implicated in IPH suggest that sex-steroid differences may account for some differences in carotid IPH presence.

The potential of menopause hormone therapy providing cardiovascular benefits remains unproven (Bhavnani & Strickler, 2005) although recent investigations demonstrate that timing of hormone therapy administration is critical to appreciate cardiovascular protection benefits. Post-menopausal administration of estradiol within six years of menopause onset may reduce progression of carotid intima-media thickness (Hodis et al., 2016), a marker of atherosclerosis that is strongly associated with cardiovascular outcomes. The relationship of post-menopause hormone therapy with plaque components using well-designed trials appears to be an avenue for future investigation.

Our results suggest that future cohort studies may be warranted to understand the role of carotid IPH and age-specific stroke rates, as well as further investigate the possibility of delayed incidence of carotid IPH in women. Prospective longitudinal studies to evaluate carotid IPH incidence rates in men and women would also clarify the natural history of carotid IPH, and affirm the role of estrogen in sex differences.

2.6 Conclusions

Males have greater age-specific odds of MRI depicted carotid IPH compared to females, and with increasing age, the odds of carotid IPH in females becomes closer to that of males. Differences in the presence of carotid IPH, an unstable plaque component, may partly explain differential stroke rates between the sexes, and further studies are warranted.
Chapter 3—Carotid Magnetic Resonance Imaging Depicted Intraplaque Hemorrhage in Embolic Stroke of Undetermined Source

This chapter contains sections or content either adapted or reproduced with permission from the following peer-reviewed literature.

3.1 Overview

**Background and Purpose:** Many embolic strokes are of undetermined source (ESUS). Carotid artery intraplaque hemorrhage (IPH), an unstable component of atherosclerosis, may be an under-recognized etiology in ESUS patients. We investigated the prevalence of carotid IPH detected non-invasively by magnetic resonance imaging (MRI). **Methods:** This pilot study analyzed data from a prospective cohort of patients with a recent ESUS who underwent MRI for carotid IPH assessment. All patients had carotid artery stenosis <50%. The primary outcome was the presence of carotid IPH ipsilateral to the cerebral ischemic event. **Results:** The cohort comprised 35 consecutive patients with a recent carotid-territory ESUS who underwent carotid MRI (mean age 74.3 ± 9.6 years). We found ipsilateral and contralateral IPH in 7/35 (20.0%) and 3/35 (8.6%) of patients, respectively (p= 0.005). **Conclusions:** In this sample of ESUS patients, one in five had carotid IPH ipsilateral to their acute infarct, as detected by vessel wall MRI. Further studies are warranted to investigate carotid IPH as an etiology of ESUS.

3.2 Introduction

Embolic stroke of undetermined source (ESUS), a non-lacunar brain infarct without a significant proximal artery stenosis or high-risk cardioembolic source, accounts for up to 25% of ischemic strokes (R. G. Hart, Catanese, Perera, Ntaios, & Connolly, 2017; Robert G. Hart et al., 2014). Large artery atherosclerotic stenosis (≥ 50%) is responsible for ≈25% of ischemic strokes (Robert G. Hart et al., 2014). Stenosis ≥ 50% has been the traditional criterion for stroke etiological classification (Adams et al., 1993). However, stenosis alone may underestimate the role of atherosclerosis due to outward luminal remodeling in early atherosclerosis (Saam et al., 2016) and provides little information on vulnerable plaque composition.

MRI of the carotid vessel wall allows characterization of plaque components. Intraplaque hemorrhage (IPH) is a plaque component of particular interest since it is associated with instability and has been linked with both incident and recurrent stroke (Hosseini et al., 2013; Saam et al., 2013; Singh et al., 2009). IPH has been reported in lower grades of stenosis (Cheung et al., 2011; Freilinger et al., 2012; Gupta et al., 2016; Gupta, Gialdini, et al., 2015) and may
represent an unrecognized source of thromboembolism in patients with ESUS. We, therefore, sought to study the presence of IPH among ESUS patients.

3.3 Materials and Methods

In this single-site retrospective study, we analyzed prospectively-collected clinical and imaging data in a sample of ESUS patients (n=60) who were enrolled in a clinical trial of cardiac rhythm monitoring conducted between February 1, 2009 and February 1, 2013 (Gladstone et al., 2014). Institutional ethics board approval was received, and informed consent was obtained.

We restricted the present analysis to a sample of patients presenting with a recent carotid-territory index cerebral ischemic event who underwent baseline MRI for the detection of carotid IPH. Carotid MRI for IPH detection was routinely acquired at our institution as part of our clinical stroke MRI protocol. All participants underwent standard diagnostic workup by a stroke neurologist with brain imaging (computed tomography (CT) and/or MRI brain), vascular imaging (magnetic resonance angiography (MRA), computed tomography angiography (CTA), and/or ultrasound), echocardiography (TTE and/or TEE), baseline 12 lead ECG and at least 24 hours of Holter ECG monitoring that did not reveal significant vessel stenosis, atrial fibrillation, or other most responsible etiological diagnosis. Exclusion criteria were patients with i) Oxfordshire classification of "posterior circulation syndrome" or "lacunar syndrome," ii) bilateral or multi-territory acute ischemia on neuroimaging, iii) any carotid stenosis ≥ 50% by ultrasound, MRI, or CTA, and iv) atrial fibrillation. The study participant flow chart is overviewed in Figure 3.1.
Clinical data were collected prospectively by a trained study coordinator. Carotid IPH imaging was acquired using a 3D T1-weighted gradient echo (GRE) black-blood pulse sequence on a 3T Philips Medical System Scanner in conjunction with a 16-channel neurovascular coil. Neuroradiologists routinely interpret carotid IPH (see Figure 3.2) at our institution. Reliability and technical pulse sequence parameters have been previously reported (A. R. Moody et al., 2003; Singh et al., 2013).

The primary outcome was the presence of carotid IPH ipsilateral to the side of cerebrovascular infarct. Descriptive statistics are reported. Fisher’s Exact test was used to assess differences in the presence of IPH ipsilateral to events. The analysis was performed using SAS Version 9.4 (SAS Institute, Cary, NC, USA).
3.4 Results

Among the ESUS cohort patients analyzed (n=35, mean age ± SD in years, 74.3 ± 9.6), no significant differences were found in characteristics between patients with and without carotid IPH (Table 3.1). No significant differences were found in the degree of carotid stenosis between patients with and without IPH (14.3% vs. 10.7%, p=1.000), and of the four patients with carotid stenosis in this cohort, one was found in ipsilateral carotid IPH patients.

Among the 35 ESUS patients, seven patients had carotid IPH (20.0%). Of the seven patients with carotid IPH, three had bilateral vessel wall disease. IPH was more common in the ipsilateral carotid artery (7/35 patients; 20.0%) than in the contralateral carotid artery (3/35 patients; 8.6%); (p=0.005). Among those with an acute infarct demonstrated by restricted
diffusion on DWI-MRI, ipsilateral and contralateral IPH was found in 4/16 patients (25.0%) vs. 3/16 patients (18.8%); (p=0.007).

**Table 3.1: Characteristics of Study Participants.**

<table>
<thead>
<tr>
<th></th>
<th>Total (n=35)</th>
<th>Carotid Intraplaque Hemorrhage (n=7)</th>
<th>No Carotid Intraplaque Hemorrhage (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD years</td>
<td>74.3 ± 9.6</td>
<td>73.1 ± 6.4</td>
<td>74.6 ± 10.4</td>
</tr>
<tr>
<td>Female, number (%)</td>
<td>19 (54.3)</td>
<td>3 (42.9)</td>
<td>16 (57.1)</td>
</tr>
<tr>
<td>Race, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>26 (74.3)</td>
<td>4 (57.1)</td>
<td>22 (78.6)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (25.6)</td>
<td>3 (42.9)</td>
<td>6 (21.4)</td>
</tr>
<tr>
<td>Modified Rankin Scale Score (&gt;2)</td>
<td>1 (2.9)</td>
<td>0 (0.0)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Vascular Risk Factors or Comorbidities, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>10 (28.6)</td>
<td>2 (28.6)</td>
<td>8 (28.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>26 (74.3)</td>
<td>7 (100.0)</td>
<td>19 (67.9)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>28 (80.0)</td>
<td>6 (85.7)</td>
<td>22 (78.6)</td>
</tr>
<tr>
<td>Prior Ischemic Stroke</td>
<td>4 (11.4)</td>
<td>0 (0.0)</td>
<td>4 (14.3)</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>7 (20.0)</td>
<td>1 (14.3)</td>
<td>6 (21.4)</td>
</tr>
<tr>
<td>Coronary Angioplasty or Stenting</td>
<td>5 (14.3)</td>
<td>1 (14.3)</td>
<td>4 (14.3)</td>
</tr>
<tr>
<td>Coronary Bypass Surgery</td>
<td>4 (11.4)</td>
<td>1 (14.3)</td>
<td>3 (10.7)</td>
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<tr>
<td>Cardiac Valvular Surgery</td>
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<td>0 (0.0)</td>
<td>1 (3.6)</td>
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<tr>
<td>Carotid Endarterectomy</td>
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<td>0 (0.0)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Carotid Stenting</td>
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<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
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<td>3 (10.7)</td>
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<tr>
<td>Patent Foramen Ovale</td>
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</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>2 (5.7)</td>
<td>1 (14.3)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Remote Smoker</td>
<td>18 (51.4)</td>
<td>2 (28.6)</td>
<td>16 (57.1)</td>
</tr>
<tr>
<td>Carotid Stenosis, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>31 (88.6)</td>
<td>6 (85.7)</td>
<td>25 (89.3)</td>
</tr>
<tr>
<td>1-49%</td>
<td>4 (11.4)</td>
<td>1 (14.3)</td>
<td>3 (10.7)</td>
</tr>
</tbody>
</table>
3.5 Discussion

Among ESUS patients, we found that one in five had carotid IPH identified by vessel wall MRI ipsilateral to the side of the index stroke event. This observation raises the possibility that embolism from unstable non-stenotic or mildly-stenotic atherosclerotic plaque may be the culprit stroke etiology in some patients.

Our study adds to sparse data on IPH as a potential stroke etiology in ESUS (Freilinger et al., 2012; Gupta et al., 2016; Gupta, Gialdini, et al., 2015). Our findings are consistent with Gupta et al. and Freilinger et al. who found IPH ipsilateral to stroke in 22% (6/27) and 28% (9/32) patients using axial 3D TOF-MRA and multi-contrast MRI, respectively. We used 3D T1 GRE fat-suppressed imaging, a highly sensitive and validated imaging method, which when first described (A. R. Moody et al., 1999) also identified 5/20 patients with acute stroke who had IPH but no significant stenosis ipsilateral to the side of cerebrovascular infarction. The 3D T1 GRE fat-suppressed imaging acquisition is quick, with a scan time of 6 minutes, and does not require contrast administration. Combined with MRA, this provides a sensitive means of assessing possible causes of stroke arising from the carotid artery including small juxtaluminal IPH which may otherwise be obscured by high signal within the lumen on TOF-MRA. The use of a standard neurovascular coil, rather than dedicated carotid coils, make this technique easily translatable into clinical practice (A. R. Moody et al., 2003; A. R. Moody & Singh, 2016).

We consider our results hypothesis-generating, requiring confirmation in larger prospective studies. Strokes were defined as cryptogenic as part of a prospective trial by a stroke neurologist; however, it is conceivable that not all stroke etiologies may have been detected since not all patients underwent transesophageal echocardiography or prolonged ECG monitoring. Furthermore, the finding of IPH may be incidental and asymptomatic, rather than etiologically linked to the index stroke event. Further research should define its prognostic significance. An a priori decision to use Fisher’s Exact test given the anticipated small sample size. Emboli from one carotid are unlikely to enter the contralateral circulation allowing an assumption of independence. In any case, secondary analysis using McNemar’s test, assuming dependence, was also found to be significant for the presence of IPH ipsilateral to ESUS (p<0.05).
In conclusion, among patients with a recent ESUS, we observed one in five had carotid IPH ipsilateral to the index acute stroke event. Further studies are warranted to investigate carotid IPH as a potential etiology of ESUS.
Chapter 4—Identifying a High-Risk Cardiovascular Phenotype by Carotid Magnetic Resonance Imaging

Depicted Intraplaque Hemorrhage

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4.1 Overview

**Background and Purpose:** Intraplaque hemorrhage (IPH), a component of late-stage complicated plaque, identified within carotid endarterectomy surgical specimens, has been recently demonstrated to predict cardiovascular (CV) events. Magnetic resonance imaging (MRI) is able to depict carotid IPH. We investigated the ability of carotid MR-depicted IPH (MR-IPH) to identify high-risk CV patients. **Materials and Methods:** From January 2008 to April 2011, 216 patients (mean age, 67.5 years; range, 31-100) referred for neurovascular MRI at an academic tertiary care center underwent 3T carotid MRI with adjunct 3D high-spatial-resolution coronal imaging to detect magnetic resonance (MR)-IPH. Five experienced neuroradiologists made a binary decision about the presence or absence of MR-IPH. Patients’ charts were reviewed blindly for demographic and CV outcomes data. **Results:** Of the patients with and without MR-IPH, 62.5% (15/24) and 19.8% (38/192) had a composite CV event (defined as a past myocardial infarction (MI), coronary intervention (i.e., angioplasty, stenting or bypass graft) and/or peripheral vascular disease), respectively. The odds ratio (OR) of a composite CV event in the MR-IPH group was 6.75 (bivariable analysis, 95% CI, 2.75-16.6, p<0.0001) and 3.25 (multivariable regression analysis, 1.14-9.37, p=0.028). MR-IPH had the highest OR of a prior CV event compared to other variables including age, sex, hypertension and stenosis. The OR of individual CV events was also significant: MI (3.35, 95% CI 2.11-14.2, p<0.01), coronary stenting (26.4, 95% CI 8.80-79.4, p<0.01), coronary angioplasty (21, 95% CI 4.84-91.1, p<0.01), and PVD (3.35, 95% CI 1.09-10.3, P<0.05). **Conclusions:** MR-IPH is independently associated with prior CV events in patients who are evaluated for neurovascular disease. Carotid MR-IPH, employed easily in routine clinical practice, is emerging as an indicator of systemic vascular disease and may potentially be a useful surrogate marker of CV risk, including in those already undergoing neurovascular imaging.

4.2 Introduction

Intraplaque hemorrhage (IPH), a component of atherosclerotic plaque, is an emerging marker of plaque instability (Freilinger et al., 2012; Lindsay et al., 2012b; A. Moody, 2012). Carotid IPH in endarterectomy surgical specimens has been recently shown to predict an individual’s risk of
future cardiovascular (CV) events, defined as a composite endpoint of vascular events and interventions (5) (Hellings et al., 2010). Similarly, in stable coronary artery disease (CAD) patients, MR-depicted IPH (MR-IPH) is associated with future CV events (6) (Noguchi, Yamada, Higashi, Goto, & Naito, 2011). Patients with neurovascular disease are at an increased risk of CV events, in some reports, even more than patients with established CAD (Steg et al., 2007). These findings support the evolving understanding that atherosclerosis is a systemic disease that commonly affects multiple vascular beds.

Patients suspected of neurovascular disease routinely undergo MRI evaluation, and MR-IPH has been suggested as a marker of future neurovascular ischemic events (Freilinger et al., 2012; Lindsay et al., 2012b; Singh et al., 2009) (Gupta et al., 2013; Saam et al., 2013). We therefore hypothesized that patients investigated for neurovascular disease found to have MR-IPH would have an increased number of prior CV events indicative of a high risk CV phenotype. Thus, this study aimed to determine the ability of MR-IPH to identify past CV events, defined as myocardial infarction (MI), coronary intervention (i.e., angioplasty, stenting, or bypass graft), and/or peripheral vascular disease (PVD), in patients investigated for neurovascular disease.

4.3 Methods

The institutional research ethics review board approved this chart review and waived the requirement for informed consent because the MR images and data were obtained as part of routine clinical care.

4.3.1 Participants

Patients referred from four stroke neurologists at our institution for MRI evaluation of neurovascular disease from January 1, 2008 to April 30, 2011 were eligible for this cross-sectional study (n=842). Patients being assessed for suspected neurovascular disease routinely undergo an MR sequence to detect carotid IPH (see Figure 4.1) at our institution.
Figure 4.1: Multiplanar Images of Carotid Intraplaque Hemorrhage within the Wall of the Left Carotid Artery. Multiplanar images (A, coronal; B, axial; c, sagittal) of carotid intraplaque hemorrhage (arrowheads) within the wall of the left carotid artery. Intraplaque hemorrhage is detected using a 3D-T1-weighted fat-suppressed fast field echo sequence by exploiting the T1 shortening effects of methemoglobin. The technique has been histologically validated. Reproduced with permission from Singh et al., 2012 (License 4071520167464).

A sample of 216 patients was selected from the total eligible participants (n= 842). The first 216 charts attained from the Health Records Department at our institution were included. The study sample size (n=216) was based on the method previously described by Eng (Eng, 2003) and used a statistical power of 0.9 (corresponding to Zpwr = 1.282), conventional significance criterion of p<0.05 (corresponding to Zcrit = 1.960), and pilot data that suggested the minimum expected absolute difference in proportion of events in the MR-IPH group to be 20%. None of the 216 subjects was excluded from the study.
4.3.2 MRI Protocol

MRI to detect carotid vessel wall IPH has been validated with similar signal features demonstrated on 1.5 Tesla and 3.0 Tesla field strengths (Kerwin et al., 2008; Underhill et al., 2008). Patients were scanned using a 3.0-Tesla Philips Medical Systems Scanner with a 16 elements neurovascular coil (Philips Achieva, SENSE-NV-16). Three-D high-spatial-resolution to detect MR-IPH was performed using a T1 weighted inversion recovery 3D Fast Field Echo sequence in the coronal plane (echo time, 4 msec; repetition time, 11 msec; matrix 512x256; flip angle 15°; field of view, 27x19 cm; number of excitations, 4; slice thickness, 0.5mm). The imaging time was 8 minutes and 54 seconds. No contrast media was used.

4.3.3 Evaluation of Intraplaque Hemorrhage

MR-IPH was defined as plaque signal intensity that exceeded the intensity of the adjacent skeletal muscle by 50% (see Figure 4.2) per the previously validated protocol (Bitar et al., 2008; A. R. Moody et al., 2003). Five experienced neuroradiologists who routinely report MR-IPH studies at our institution made a binary decision on the presence or absence of IPH. Increased signal intensity was only considered to be carotid IPH if it was in the carotid artery wall; hyper-intensities elsewhere were not considered.
Figure 4.2: Multiplanar Images Showing Intraplaque Hemorrhage in the Left Carotid Artery. Multiplanar images (A, coronal; B, axial; C, sagittal) showing intraplaque hemorrhage (arrowheads) in the left carotid artery. Intraplaque hemorrhage is considered when high signal intensity is seen in the carotid artery wall and is greater than 150% compared to the adjacent skeletal muscle. Reproduced with permission from Singh et al., 2012 (License 4071520167464).

4.3.4 Data Collection and Definition of Outcomes

Patients’ electronic and paper charts were reviewed. The initial stroke neurologists’ consultation letters were used to collect study subjects’ baseline data and past medical history of vascular events. MR angiography was used to determine degree of carotid stenosis. A per patient design was used—patients with one or both arteries with a stenosis greater than 30% were considered to have a stenosis greater than 30% for the purpose of data analysis. A 30% cut-off was selected to overestimate the value of stenosis in determining CV events (i.e., provide a conservative measure of the role of MR-IPH in determining CV events when stenosis is controlled for). MR-IPH status
was similarly defined as the presence of MR-IPH in one or both arteries. A composite CV event was defined as prior MI, and/or CV intervention (stenting, angioplasty, coronary artery bypass graft), and/or PVD.

4.3.5 Statistical and Data Analysis

Statistical analysis was performed by a biostatistician using SAS Version 9.2 (SAS Institute, Cary, NC, USA). A \( p \)-value of less than 0.05 was considered to indicate a significant difference. Descriptive statistics were calculated for all variables of interest. Continuous variables were summarized using means and standard deviations, whereas categorical variables were summarized using counts and percentages.

Categorical baseline variables were compared using chi-square and Fisher’s exact tests between patients with and without MR-IPH. Continuous variables were compared using two sample two-sided t-tests between the groups. Multivariable logistic regression analysis, adjusted for risk factors significantly different between the MR-IPH groups, was performed to evaluate whether MR-IPH was an independent risk factor for CV events. Prior to analysis, predictor variables were assessed for the presence of multicollinearity. A tolerance statistic less than 0.4 was considered to indicate the presence of multicollinearity, and in such cases, only one member of a correlated set was retained for the multivariable model. Predictor estimates were reported as odds ratios (OR) and their associated 95% confidence intervals (CI).

4.4 Results

All 216 patients (mean age, 67.5 years ± 14.60 [standard deviation]; range, 31-100) referred for MRI evaluation of neurovascular disease were included for analysis. Of the 216 patients, 11.1% (24/216) had MR-IPH in either one or both carotid arteries.

Patients with MR-IPH were 8.1 years older than patients without MR-IPH (mean age, 66.6±14.8 years; range, 53-100 vs. 74.7±11.1; range, 31-91). Compared to patients without MR-IPH, patients with MR-IPH had a significantly greater proportion of males, history of hypertension, and smokers (see Table 4.1). MR-IPH patients also had a significantly higher proportion of current users of statins and antiplatelets. Otherwise, no significant differences were noted between the groups in demographic variables.
Table 4.1: Demographic Characteristics of Participants by the Presence of Magnetic Resonance Depicted Intraplaque Hemorrhage.

<table>
<thead>
<tr>
<th></th>
<th>Patients with MR-IPH (n=24)</th>
<th>Patients without MR-IPH (n=192)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (mean±/- sd)</td>
<td>74.7±11.1</td>
<td>66.6±14.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Female Sex, number (%)</td>
<td>4 (16.7)</td>
<td>109 (56.8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Carotid Stenosis &gt; 30%, number (%)</td>
<td>18 (75)</td>
<td>40(20.8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Patient History, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>21 (87.5)</td>
<td>119 (62.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>6 (25)</td>
<td>35 (18.2)</td>
<td>0.41</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>17 (70.8)</td>
<td>110 (57.3)</td>
<td>0.20</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>3 (12.5)</td>
<td>20 (10.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Smoking</td>
<td>16 (66.7)</td>
<td>70 (36.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Alcohol</td>
<td>2 (8.3)</td>
<td>19 (9.9)</td>
<td>0.99</td>
</tr>
<tr>
<td>Cardiovascular Family History</td>
<td>5 (20.8)</td>
<td>53 (27.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Medication, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (70.8)</td>
<td>111 (57.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>7 (29.2)</td>
<td>27 (14.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>Statin</td>
<td>19 (79.2)</td>
<td>110 (57.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>17 (70.8)</td>
<td>123 (64.1)</td>
<td>0.51</td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>11 (45.8)</td>
<td>43 (22.4)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

MR-IPH indicates magnetic resonance imaging depicted intraplaque hemorrhage. P values for normally distributed continuous variables are from two sample two-sided t tests (age). P values for categorical variables are from the X² test (female sex, carotid stenosis >30%, hypertension, dyslipidemia, smoking, cardiovascular family history, hypertension medication, diabetes mellitus medication, statin medication, anticoagulant medication, antiplatelet medication) or Fisher’s Exact Test (diabetes mellitus, atrial fibrillation, alcohol).

Of the patients with and without MR-IPH, 62.5% (15/24) and 19.8% (38/192) had a composite CV event, respectively (see Table 4.2). The odds ratio of a composite CV event in the MR-IPH group was 6.75 (95% CI, 2.75-16.6, p< 0.0001). The odds ratio of each of PVD, MI, coronary stenting, and coronary angioplasty was also greater in the MR-IPH group.
Table 4.2: Cardiovascular Events by the Presence of Magnetic Resonance Depicted Intraplaque Hemorrhage.

<table>
<thead>
<tr>
<th>Event</th>
<th>Patients with MR-IPH n=24 (%)</th>
<th>Patients without MR-IPH n=192 (%)</th>
<th>P-value</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite Cardiovascular Outcome</td>
<td>15(62.5)</td>
<td>38(19.8)</td>
<td>&lt;0.01</td>
<td>6.75</td>
<td>2.75—16.6</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td>5(20.8)</td>
<td>14(7.29)</td>
<td>&lt;0.05</td>
<td>3.35</td>
<td>1.09—10.3</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>9(37.5)</td>
<td>19(9.90)</td>
<td>&lt;0.01</td>
<td>5.46</td>
<td>2.11—14.2</td>
</tr>
<tr>
<td>Coronary Stenting</td>
<td>12(50.0)</td>
<td>7(3.65)</td>
<td>&lt;0.01</td>
<td>26.4</td>
<td>8.80—79.4</td>
</tr>
<tr>
<td>Coronary Angioplasty</td>
<td>6(25.0)</td>
<td>3(1.56)</td>
<td>&lt;0.01</td>
<td>21.0</td>
<td>4.84—91.1</td>
</tr>
<tr>
<td>Coronary Artery Bypass Graft</td>
<td>2(8.33)</td>
<td>13(6.77)</td>
<td>0.68</td>
<td>1.25</td>
<td>0.27—5.92</td>
</tr>
</tbody>
</table>

MR-IPH indicates magnetic resonance imaging depicted intraplaque hemorrhage. All p-values are for categorical variables from the X2 test.

Bivariable and multivariable regression analysis models utilized demographic variables that were significantly different in the group with and without MR-IPH and included age, sex, stenosis greater than 30%, hypertension, and smoking (see Table 4.1). The bivariable analysis of these variables (i.e., known risk factors) demonstrated that each of the variables was independently associated with the composite cardiovascular outcome (see Table 4.3). The multivariable regression analysis demonstrated that MR-IPH, male sex, and hypertension were most associated with the composite CV event (see Table 3.3). The odds ratio of a composite CV event in the MR-IPH group using a multivariable regression analysis was 3.26 (1.14-9.37, p=0.028), making it the most significant risk factor.
Table 4.3: Odds Ratios of a Composite Cardiovascular Event.

<table>
<thead>
<tr>
<th></th>
<th>Bivariable, Odds Ratio (95% CI)</th>
<th>P-value</th>
<th>Multivariable, Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR-IPH</td>
<td>6.75(2.75—16.6)</td>
<td>&lt;0.01</td>
<td>3.26(1.14—9.37)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age</td>
<td>1.04 (1.01—1.06)</td>
<td>&lt;0.01</td>
<td>--</td>
<td>0.19</td>
</tr>
<tr>
<td>Male Sex</td>
<td>3.03 (1.56, 5.88)</td>
<td>&lt;0.01</td>
<td>2.44 (1.19, 5.00)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Carotid Stenosis &gt;30%</td>
<td>2.50(1.29—4.84)</td>
<td>&lt;0.01</td>
<td>--</td>
<td>0.46</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.41(1.56—7.46)</td>
<td>&lt;0.01</td>
<td>2.41(1.02—5.74)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.35(0.72—2.52)</td>
<td>0.35</td>
<td>--</td>
<td>0.84</td>
</tr>
</tbody>
</table>

MR-IPH indicates magnetic resonance imaging depicted intraplaque hemorrhage. The adjusted odds ratios of MR-IPH after adjustment for characteristics that significantly differed between the groups at baseline are shown. The bivariable odds ratio depicts the independent effect of the variable on composite cardiovascular outcome; the multivariable odds ratio controls for all variables (MR-IPH, age, sex, stenosis, hypertension, and smoking) in the model.

4.5 Discussion

This study demonstrates the ability of carotid MR-IPH to identify patients with a high risk CV phenotype. Patients with MR-IPH have greater odds of having a history of MI, coronary intervention, and/or PVD (i.e., patients considered to be high CV risk). The study suggests that carotid intraplaque hemorrhage, a feature of late-stage complicated plaque, may be a surrogate marker for systemic vascular disease, both CV disease and PVD.

Two imaging studies have shown a similar association in coronary artery disease patients (Noguchi et al., 2011; X. Zhao, Underhill, & Zhao, 2011), but no studies have investigated this relationship in patients selected because of their neurovascular disease. Such patients are an ideal population for MR-IPH screening because the cohort is known to be at risk of CV events (Steg et al., 2007) and their carotid arteries are routinely investigated in clinical practice with MRI. The addition of a single sequence to a comprehensive neurovascular examination, adding only 8 minutes and 54 seconds to the scan time to assess for carotid MR-IPH, is both time and cost efficient. Carotid MR-IPH is also practical to implement into routine clinical practice in the evaluation of neurovascular patients; our study demonstrates the routine clinical use of MR-IPH over a four-year period.
In this study of suspected neurovascular patients, MR-IPH was associated with prior CV events, independent of degree of carotid stenosis and other established risk factors including age, male sex, hypertension, and smoking, using a multivariable regression model. The findings highlight that MR-IPH may be a better marker of plaque instability because it is associated with prior CV events even when controlled for known predictors of CV outcomes. Also, the ability to associate MR-IPH in a carotid artery with cardiac events suggests a common underlying pathophysiology of systemic atherosclerosis.

Several recent studies have also demonstrated the emerging relationship between carotid MR-IPH and plaque instability. In addition to the association of MR-IPH with CV outcomes, carotid MR-IPH has been associated with various neurovascular outcomes. The presence of carotid IPH has been demonstrated in varying degrees of stenosis (Cheung et al., 2011), and the presence of complicated atheroma has been recently reported in a symptomatic population with low-grade stenosis (Yoshida et al., 2012). Low-grade carotid stenosis patients with MR-IPH may therefore potentially benefit from being considered of a high risk CV phenotype, despite the lack of significant carotid disease defined by stenosis.

A significant advantage of this study is that the data were obtained in a routine clinical setting in a population that could benefit from being identified with a high risk CV phenotype. Using a routinely screened population also resulted in a sufficient study sample size to allow for multivariable regression model analysis controlling for established CV risk factors. The MR-IPH technique provides the relevant information by exploiting the generation of endogenous contrast, thus not requiring the added cost and risks of injected exogenous contrast agents. The use of a 3-D technique makes acquisition simple, and post-processing can provide vascular cross-sectional images regardless of the tortuosity of the vessel under examination. Although the development of MR-IPH techniques to visualize the coronary arteries is in progress, imaging the carotid artery vascular bed is advantageous since it is a relatively spared cardiorespiratory artifact. Carotid MR-IPH has good inter-observer and intra-observer reliability, as well as good inter-scan reliability.

This study has limitations. The prior events for each individual relied on retrospective chart review. Events were clinically defined using additional diagnostic data such as ECG and
blood work. A convenience sample was used. Of the total eligible patients, the first 216 returned by the Health Records Department at our institution were reviewed. Ideally, a prospective population outcome study of subsequent patients referred for investigation in this cohort would allow for a more vigorous assessment of the ability of MR-IPH to identify individuals with a high risk CV phenotype.

This study has shown the ability to retrospectively phenotype patients being investigated for possible neurovascular disease using MRI. While the clinical information provided is already known from the patients’ clinical histories, the study provides proof of the principle that IPH can act as a systemic vascular phenotypic marker. If confirmed prospectively, the presence of IPH detected while imaging the carotid vessels, could therefore be used to identify which patients are at an increased risk of having occult, but significant, CV disease. Detection of MR-IPH would then provide an opportunity to detect and potentially treat such disease before it becomes symptomatic, avoiding subsequent morbidity and mortality and their associated societal costs.

The results also raise the possibility of expanding the use of carotid MR-IPH outside of the realm of investigating neurovascular disease to identify high CV risk groups. The possibility may therefore exist of screening known high-risk populations by the simple application of one MRI sequence. To make this cost effective, this would likely need to be combined with simple and cheap pre-testing, perhaps using blood tests that would allow the identification of smaller groups of high risk individuals in already known vulnerable populations (e.g., diabetic patients).

MR-IPH may stratify prior CV risk in patients being assessed by MR for neurovascular disease. The systemic nature of atherosclerosis, the clinical and research need to identify the vulnerable patient at risk of CV events, and increasing realization of the importance of plaque characteristics compared to stenosis all make in-vivo carotid MR-IPH a clinically promising marker for individual CV risk phenotyping.

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5.1 Overview

**Background and Purpose:** Addition of a 3D T1w gradient echo (GRE) black-blood acquisition to routine 3D time-of-flight (TOF) provides an opportunity to evaluate both vessel wall and intraplaque hemorrhage volume, an emerging predictive marker of stroke. We investigate inter-, intra-, and scan-rescan reliability of this 3D carotid artery magnetic resonance imaging (MRI) approach and assess agreement with 2D T1 MRI. **Materials and Methods:** Consecutive patients (n=22) were recruited from a carotid artery clinical trial including 3D T1w GRE black-blood, 3D TOF, and 2D MRI. Coregistered 3D and 2D axial images were created to assess vessel wall volume agreement. Inter- and intra-rater reliability of vessel wall and intraplaque hemorrhage volumes for 3D axial images and vessel wall volume for 2D axial images were additionally evaluated. Scan-rescan reliability of vessel wall and intraplaque hemorrhage volumes was evaluated in patients with repeat 3D MRI. Analysis included intra-class correlation coefficients and Bland and Altman plots. **Results:** Three-D carotid artery MRI had excellent reliability for the volumetric quantification of vessel wall (ICC\textsubscript{intra}=.859, ICC\textsubscript{inter}=.881, ICC\textsubscript{scan-rescan}=.928), and intraplaque hemorrhage (ICC\textsubscript{intra}=.998, ICC\textsubscript{inter}=.996, ICC\textsubscript{scan-rescan}=.949). No significant difference was found in vessel wall volume on 3D compared to 2D carotid artery MRI (mean bias, 66 ± 367 mm$^3$, p=0.13), and both methods had excellent absolute agreement (ICC\textsubscript{3D-2D}=0.874, 95% CI 0.709 to 0.948, p<0.01). **Conclusions:** Addition of a 3D T1w GRE black-blood acquisition to routine 3D TOF is a highly reliable approach for the volumetric quantification of vessel wall and intraplaque hemorrhage. This approach had excellent agreement with 2D T1 MRI for quantification of vessel wall volume.

5.2 Introduction

Carotid artery vessel wall (VW) volume is a surrogate marker for atherosclerosis burden used for cardiovascular risk stratification and allows for evaluation of changes in disease status. Carotid intraplaque hemorrhage (IPH), a complicated plaque component, is an emerging marker of stroke (Singh et al., 2009; Singh et al., 2012; Takaya et al., 2006), myocardial infarction (Hellings et al., 2010; Noguchi et al., 2011; Singh et al., 2013), and progression of atherosclerosis (Sun et al., 2012; Takaya et al., 2005). Carotid artery VW volume and IPH are
emerging quantitative biomarkers under investigation in multicenter carotid atherosclerosis trials.

Evaluation of carotid VW and IPH typically requires both 2D and 3D MRI acquisitions (T. Saam et al., 2005; Touze et al., 2007) because 2D carotid artery MRI acquisitions are used to evaluate VW (Touze et al., 2007; Yuan, Beach, Smith, & Hatsukami, 1998) and 3D gradient echo sequences are used to evaluate lumen and IPH (Ota, Yarnykh, et al., 2010; J. Wang et al., 2013). Quantitative evaluation of VW and IPH using only 3D MRI would allow for better image registration due to improved spatial alignment and positioning of serial acquisitions. Three-D carotid artery MRI also results in higher spatial resolution and isotropic voxels and may improve accuracy of quantitative evaluation.

Three-D T1-weighted (T1w) gradient echo (GRE) black-blood (BB) sequences have been validated for the detection of carotid IPH (Niranjan Balu, Chu, Hatsukami, Yuan, & Yarnykh, 2008; N. Balu et al., 2011; Bitar et al., 2008; Fan et al., 2014; McNally et al., 2012; A. R. Moody et al., 2003; Ota, Yarnykh, et al., 2010; J. Wang et al., 2013; Zhu, Ferguson, & DeMarco, 2008). The sequences also allow for visualization of the outer vessel wall. Three-D TOF, routinely included in clinical neurovascular protocols, delineates the lumen. Together the sequences therefore provide an opportunity to volumetrically quantify both VW and IPH using only 3D carotid artery MRI. However, the use of a combined approach is yet to be investigated for the volumetric quantification of VW and IPH.

We hypothesized that volumetric quantification of VW and IPH is highly reliable on 3D carotid artery 3T MRI, and quantification of VW has excellent agreement with 2D carotid artery MRI. Our purpose was to investigate the inter-, intra-, and scan-rescan reliability of 3D carotid artery 3T MRI, using a 3D T1w GRE BB and TOF acquisition to volumetrically quantify vessel wall and IPH and assess agreement with 2D T1 MRI for measuring vessel wall volume.

5.3 Methods

Institutional review board approval and written informed consent were obtained for this prospective HIPAA-compliant study.
5.3.1 Study Population

Consecutive participants were recruited from the carotid imaging core site of an ongoing multicenter carotid 3T MRI trial (Tardif et al., 2013) of patients with nonsurgical carotid disease. Participants were eligible for this reproducibility study if both 3D (T1w GRE BB and TOF) and 2D carotid imaging was performed at baseline or repeat 3D imaging was performed within the four months prior to the baseline 3D trial imaging (see Figure 5.1). Patients with 3D and 2D imaging available at baseline are referred to as group 1. Patients with 3D imaging at the two time-points are referred to as group 2.

![Figure 5.1: Summary of Reliability and Agreement of 3D IPH Imaging Study Participants.](image)

5.3.2 MRI Protocol

All scans were completed on a 3T MR scanner (Philips Achieva). A dedicated 16-channel neurovascular coil (16-NV-SENSE) was used to improve spatial resolution. Carotid MRI acquisition parameters are summarized in Table 5.1. The 3D BB imaging sequence was performed using a noncardiac gated T1 fat-suppressed (using a selected water excitation RF pulse) fast field echo sequence, with imaging acquired in the coronal plane (see Figure 5.2) (repetition time (TR), 11 msec; echo time (TE), 4 msec; field of view (FOV), 270x190 mm²; matrix, 512x256 mm²; slices, 100; through-plane thickness, 0.5; voxel size 0.5 mm x 0.7 mm x 0.5 mm).
Shimming was prescribed for a 10 cm region centered over the neck to ensure B₀-field homogeneity around the carotid arteries. The field of view of 3D T₁w GRE BB acquisition provided coverage from the top of the aortic arch to the Circle of Willis. Three-D TOF imaging was acquired in the axial plane (TR, 26 msec; TE, 3.5 msec; field of view, 190x190 mm²; matrix, 360x232 mm²; slices, 160 slices; through-plane thickness, 0.7 mm; voxel size 0.5 mm x 0.8 mm x 0.7 mm). Scan times for 3D T₁w GRE BB and 3D TOF were 8:54 and 5:59 minutes, respectively. Two-D imaging was performed acquiring 16 two-mm thick slices centered at the index carotid bifurcation, resulting in a total of 3.2 cm coverage in the z-axis. Two-D T₁ acquisitions were cardiac gated and used a double inversion recovery turbo spin echo fat suppressed (using spectral presaturation with inversion recovery (SPIR)) technique, with
imaging acquired in the axial plane (TR, 833 msec; TE, 833 msec; field of view, 130x130 mm²; matrix, 256x220 mm², through-plane thickness, 2mm; pixel size, 0.5 mm x 0.6 mm ). The scan time for 2D T1 was 5:20 minutes.

**Table 5.1: Carotid Magnetic Resonance Imaging Acquisition Parameters.**

<table>
<thead>
<tr>
<th>Plane of Acquisition</th>
<th>3D T1w GRE BB</th>
<th>3D TOF</th>
<th>2D T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronal</td>
<td>Nongated</td>
<td>Nongated</td>
<td>Gated</td>
</tr>
<tr>
<td>Slice thickness, mm</td>
<td>0.5</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>Number of slices</td>
<td>100</td>
<td>160</td>
<td>16</td>
</tr>
<tr>
<td>Matrix (mm²)</td>
<td>512 x 256</td>
<td>360 x 232</td>
<td>256 x 220</td>
</tr>
<tr>
<td>Field of view (mm²)</td>
<td>270 x 190</td>
<td>190 x 190</td>
<td>130 x 130</td>
</tr>
<tr>
<td>TE (ms)</td>
<td>4</td>
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<td>TR (ms)</td>
<td>11</td>
<td>26</td>
<td>833</td>
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<tr>
<td>TI (ms)</td>
<td>800</td>
<td>None</td>
<td>600</td>
</tr>
<tr>
<td>Flip Angle (°)</td>
<td>15</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>TSE Factor</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Scan time (mins)</td>
<td>8:54</td>
<td>5:59</td>
<td>5:20</td>
</tr>
</tbody>
</table>

5.3.3 MRI Post-processing

Reslicing, registration, and segmentation were carried out using VesselMASS (Version 2014, Medis Medical Imaging Systems BV, Leiden, the Netherlands) (van 't Klooster, de Koning, et al., 2012; van ‘t Klooster, Patterson, et al., 2013; van ‘t Klooster, Staring, et al., 2013). Post-processing of each carotid resulted in the creation of 16 two-mm thick slices per acquisition from the original 3D data. For group 1 patients, using VesselMASS, 3D T1w GRE BB, and 3D TOF data was registered and resliced to 2-mm thick axial images, corresponding to the 2D T1 acquisition. Figure 5.3 demonstrates the axial T1w GRE BB images created that correspond to the 2D T1 reference.
Figure 5.3: Post-processing of Patients with 3D and 2D Imaging. Corresponding 2D T1 (a) and 3D T1w gradient echo black blood (GRE BB) (b) axial images of the left carotid artery in a patient with an eccentric atherosclerotic plaque are shown. Three-D T1w GRE BB imaging highlights presence of intraplaque hemorrhage. The 3D T1w GRE BB sequence improves differentiation of intraplaque hemorrhage from other plaque components within the vessel wall. Three-D T1w GRE BB Imaging is also more sensitive for detection of intraplaque hemorrhage, allowing for identification of small amounts (arrowheads). © Navneet Singh and Alan Moody, 2016. All rights reserved.

For group 2 patients, using VesselMASS, 3D T1w GRE BB, and 3D TOF data was resliced into 16 2-mm thick axial images centered around the carotid bifurcation (since corresponding 2D T1 reference images were not acquired for these patients) (see Figure 5.1). 5.3.4 Vessel Wall Segmentation

For the 3D MRI acquisitions, the lumen was contoured using an automated MRA segmentation function after indicating the center of the lumen on the first and last slice on the axial TOF images. The outer wall of the vessel was contoured on the axial T1w GRE BB images using an automatic VW segmentation function. For 2D T1 images, the lumen and outer wall were both
automatically contoured using the VW segmentation function after indicating the center of the lumen on the first and last slice. Manual review of contours and registration was made, and adjustments were made as required to ensure accurate segmentation.

5.3.5 Intraplaque Hemorrhage Segmentation

On the axial T1w GRE BB images, the primary slice distal to the flow divider was identified. A region of interest (ROI) was identified on the sternocleidomastoid (ROI area, 20 ± 5 mm²) at the same level as the carotid artery in the direction of frequency encodes (anterior-posterior) to determine signal intensity. A signal intensity of 1.5 times the ROI was used as a threshold to identify IPH in the VW. Classifying presence of IPH by detecting signal intensity greater than 1.5 times the intensity of the sternocleidomastoid is accepted to determine the presence of IPH (Cheung et al., 2011; Hosseini et al., 2013; A. R. Moody et al., 2003; Murphy et al., 2003; Singh et al., 2009; Singh et al., 2013). A ROI was drawn around the outer VW boundary, and a signal above the threshold was included within the boundary (see Figure 5.4).

Figure 5.4: Segmentation of Intraplaque Hemorrhage and the Vessel Wall. Corresponding 2 mm axial images of 2D T1 (a), 3D T1w gradient echo black blood imaging (b) and 3D time-of-flight (c) with the results of semi-automated contouring of the carotid vessel wall shown (lumen, red; outer wall, green). The
shaded blue region (b) within the vessel wall highlights the intraplaque hemorrhage greater than 1.5 times the signal intensity of the adjacent sternocleidomastoid (white). © Navneet Singh and Alan Moody, 2016. All rights reserved.

5.3.6 MRI Review

Evaluation of 16 axial two-mm images per carotid artery was made by two independent readers (N.S., T.M.). Carotid VW and IPH volume were measured for each image. Total carotid volumes of VW and IPH were determined by VesselMASS by summation of the 16 axial slices per carotid. Images were segmented at least two weeks a part. One rater resegmented all the images after a further two weeks (N.S.). Each set of 16 comparisons was reviewed in a randomized order. Raters were blinded to patient information and the results of prior reads.

5.3.7 Statistical Analysis

Descriptive statistics were reported with mean ± SD or median for continuous variables and as percentages for categorical variables. An independent samples t-test was used to compare normally distributed means, and the F-test was used to confirm equal variances.

Intra-rater reliability (N.S. 1 vs. N.S. 2), inter-rater reliability (N.S. 1 vs. T.M.), scan-rescan reliability (N.S. 1 vs. N.S. 1), and agreement (N.S. 3D vs. N.S. 2D) were assessed with single measure intra-class correlation coefficients (ICCs). Absolute type agreement was reported for all measures except for outer wall and lumen measurements comparing 3D and 2D methods. Interpretation of ICC was defined as poor reproducibility (ICC < 0.4), fair to good reproducibility (0.4 ≤ ICC < 0.75), and excellent reproducibility (≥ 0.75) (Rosner, 2010). Bland and Altman (B-A) plots were also used to depict measurement reliability of 3D carotid artery MRI, as well as agreement between 3D and 2D carotid artery MRI. Statistical analysis was performed using SPSS (SPSS Version 13.0, Chicago, IL, USA) and MedCalc (MedCalc Version 13.0, Ostend, Belgium).

5.4 Results

Demographic characteristics of the included study (n=16) participants (age, 74 ±10.8 years) are summarized in Table 5.2. Six of the 22 study patients were excluded due to motion artifact (n =
4) and improperly acquired 2D imaging (n = 2). Additionally, a single carotid artery from each of two patients in group 2 was excluded due to noninterpretable quality and a carotid endarterectomy between scan and re-scan, resulting in 10 carotids (from six patients) available for comparison in group 2. The majority of patients studied were asymptomatic (93%, 15/16), male (75%, 12/16), hypertensive (93%, 15/16), dyslipidemic (62.5%, 10/16), and smokers (66.7%, 11/16). VW volumes of carotid arteries ranged from 746 to 2444 mm$^3$. Mild, moderate, and severe carotid stenosis in the index carotid artery was present in 18.7%, 75%, and 6.3% of patients, respectively.

**Table 5.2:** Demographic Characteristics of Study Participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (mean± SD)</td>
<td>74 ± 10.8</td>
</tr>
<tr>
<td>Symptomatic, number (%)</td>
<td>1 (6.2)</td>
</tr>
<tr>
<td>Degree of Stenosis, number (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>3 (18.7)</td>
</tr>
<tr>
<td>50 to 79</td>
<td>12 (75)</td>
</tr>
<tr>
<td>80-99</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>Male Sex, number (%)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Hypertension, number (%)</td>
<td>15 (93.7)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>Smoking</td>
<td>11 (66.7)</td>
</tr>
<tr>
<td>Family History, Cardiovascular Disease</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Medication History, number (%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (93.7)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>3 (18.7)</td>
</tr>
<tr>
<td>Statin</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>1 (6.2)</td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>3 (18.7)</td>
</tr>
</tbody>
</table>
5.4.1 Intra- and Inter-Rater Reliability

Intra- and inter-rater ICCs for the volumetric quantification of the VW and IPH on 3D carotid artery MRI ranged from .859 to .998 (p<0.01) and are summarized in Table 5.3. Similar ICCs ranging from .947 to .997 (p<0.01) resulted from evaluation of volumes on 2D carotid artery MRI.

Figure 5.5 shows B-A plots of intra-rater and inter-rater reliability of the volumetric quantification of VW and IPH from 3D carotid artery MRI. A mean bias of -7.4 mm$^3$ for volume of IPH was found among intra-rater measurement (95% CI, -13.2 to -1.60, p=0.02). Otherwise, no statistically significant bias was observed for intra- and inter-rater measurements. No statistically significant Pearson correlations were observed between differences and means.

Figure 5.5: Bland and Altman Plots of the Intra-rater (a) and Inter-rater (b) for the Volumetric Quantification of Vessel Wall and Intraplaque Hemorrhage with 3D Carotid Artery Magnetic Resonance Imaging Using a Combined 3D T1w Gradient Echo Black Blood and 3D Time-of-flight Approach.
5.4.2 Scan-Rescan Reliability

Scan-rescan reliability (average scan-rescan, 58.2 days, range 7 to 100 days) ICCs for volumes of lumen, outer wall, VW and IPH on 3D carotid artery MRI ranged from .928 to .994 and are summarized in Table 5.3. No significant bias was observed for scan-rescan measurements.

Table 5.3: Reliability of 3-Dimensional and 2-Dimensional Carotid 3-Tesla Magnetic Resonance Imaging for the Volumetric Quantification of Vessel Wall and Intraplaque Hemorrhage.

<table>
<thead>
<tr>
<th></th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-Rater</td>
</tr>
<tr>
<td><strong>Lumen</strong></td>
<td></td>
</tr>
<tr>
<td>3D Carotid Artery MRI</td>
<td>.988 (.971 to .996)</td>
</tr>
<tr>
<td>Outer Wall</td>
<td>.959 (.899 to .984)</td>
</tr>
<tr>
<td>Vessel Wall</td>
<td>.859 (.683 to .941)</td>
</tr>
<tr>
<td>Intraplaque Hemorrhage</td>
<td>.998 (.992 to .999)</td>
</tr>
<tr>
<td><strong>2D Carotid Artery MRI</strong></td>
<td></td>
</tr>
<tr>
<td>Lumen</td>
<td>.991 (.976 to .996)</td>
</tr>
<tr>
<td>Outer Wall</td>
<td>.997 (.991 to .999)</td>
</tr>
<tr>
<td>Vessel Wall</td>
<td>.994 (.984 to .998)</td>
</tr>
</tbody>
</table>

5.4.3 3D vs. 2D carotid artery MRI

Excellent absolute vessel wall volume agreement was found between 3D and 2D carotid artery MRI (ICC=0.859, 95% CI, 0.683 to 0.941). ICCs for the agreement of 3D and 2D carotid artery MRI for the volumetric quantification of VW are summarized in Table 5.4.
Table 5.4: Agreement of 3-Dimensional and 2-Dimensional Carotid Artery Magnetic Resonance Imaging for the Volumetric Quantification of Vessel Wall.

<table>
<thead>
<tr>
<th>Volume</th>
<th>3D carotid artery MRI, mean ± SD</th>
<th>2D carotid artery MRI, mean ± SD</th>
<th>Bias, mean ± SD</th>
<th>P-value</th>
<th>ICC (95% CI), 3D vs. 2D carotid artery MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen, mm$^3$</td>
<td>1234 ± 333</td>
<td>1053 ± 273</td>
<td>181 ± 127</td>
<td>&lt;0.01</td>
<td>.913 (.795 to .965)$^a$</td>
</tr>
<tr>
<td>Outer Wall, mm$^3$</td>
<td>2804 ± 576</td>
<td>2557 ± 569</td>
<td>247 ± 164</td>
<td>&lt;0.01</td>
<td>.956 (.899 to .984)$^a$</td>
</tr>
<tr>
<td>Vessel Wall, mm$^3$</td>
<td>1569 ± 377</td>
<td>1503 ± 392</td>
<td>66 ± 187</td>
<td>0.13</td>
<td>.882 (.725 to .951)$^b$</td>
</tr>
</tbody>
</table>

$^a$Consistency type agreement $^b$Absolute type agreement

Figure 5.6 shows B-A plots of 3D versus 2D carotid artery MRI depicting the agreement of the two acquisitions for the volumetric quantification of VW. A statistically significant mean bias was observed in 3D versus 2D acquisitions for the volumes of lumen (181±127, p<0.01) and outer wall (247±164 mm$^3$, p<0.01). The mean bias of vessel wall volume was not found to be statistically significant between the two methods (66±367 mm$^3$, p=0.13).
5.5 Discussion

Addition of a 3D T1w GRE BB acquisition to routine 3D TOF was found to be highly reliable for volumetric quantification of VW and IPH, and also demonstrated excellent agreement with 2D T1 MRI for measuring VW volume. The approach allowed for the provision of only 3D carotid artery MRI to quantify VW, with 3D T1w GRE BB acquisition for outer VW delineation and 3D TOF for lumen delineation. Use of a 3D T1w GRE BB acquisition allowed for the simultaneous benefit of reliably identifying and quantifying IPH, an emerging marker of stroke.

Excellent intra-rater, inter-rater, and scan-rescan reliability of 3D carotid artery MRI volumetric quantification of VW were found. The reliability of VW, lumen, and outer wall measurements in our study all had ICCs of greater than 0.85 and were similar to multicontrast
studies assessing inter- and intra-rater reliability on 1.5T (T. Saam et al., 2005; Touze et al., 2007) and scan-rescan reliability at 3T (Li et al., 2010).

Volumetric quantification of the VW on 3D carotid artery MRI had excellent absolute agreement with 2D carotid artery MRI. However, comparison of absolute differences of OW and lumen revealed that 3D carotid artery MRI consistently overestimated compared to 2D MRI. Since both OW and lumen were overestimated, the VW volume, which is calculated by subtracting volumes of lumen from OW, resulted in no VW volume difference and excellent absolute agreement. The overestimation of both VW and lumen may be due two separate reasons. First, the absence of cardiac gating on 3D carotid artery MRI resulting from averaging the systolic and diastolic cardiac phases, compared to 2D acquisition which is gated to end diastole. An increase in vessel diameter on 3D carotid artery MRI of 1 to 2 pixels (0.5 to 1 mm) in the absence of cardiac gating has been previously reported (Sarkar, Moody, & Leung, 2009). Since the gating affects both lumen and OW proportionally, comparisons of VW on 3D carotid MRI and 2D carotid MRI can be made for VW. The second reason may be due to the difference in voxel size in the x-y dimensions. The 3D TOF, 3D T1w GRE BB, and 2D T1 sequences have x-y dimensions of 0.5x0.8, 0.5x0.7 and 0.5x0.6, respectively. We, however, advocate making comparisons on the same modality whenever possible or mathematically adjusting for any differences between two methods using calibration with phantoms.

The excellent reliability of IPH measurements in our study is likely due to assessment of IPH relying only on a single gradient echo sequence and using semi-automated methods for image processing and analysis. Intra- and inter- observer reliability of IPH measurements have been reported with ICCs of 0.60 to 0.74 with multicontrast evaluation at 1.5T (T. Saam et al., 2005; Touze et al., 2007). Use of multicontrast methods requires review of multiple image weightings (e.g., T1, TOF, T2 and PD) for determination of IPH and thus requires increased acquisitions and post-processing time. Carotid IPH at 3T is also detected with more sensitivity on 3D rapid gradient echo sequences, compared to T1-weighted fast-spin echo and 3D TOF sequences (Ota, Yarnykh, et al., 2010).

The strengths of our study included recruitment of patients with a wide distribution of
VW volumes ranging from 746 to 2444 mm³, thus demonstrating the reproducibility of 3D carotid artery MRI in patients with variable amounts of disease. The use of 3D T1w GRE BB sequence in the 3D carotid artery MRI method was also advantageous for several reasons. The simultaneous assessment of VW and IPH on 3D carotid artery MRI was made possible by the addition of a single sequence to 3D TOF that is routinely included in clinical protocols assessing carotid disease. Through-plane resolution on the order of 0.5 mm in contrast to 2 mm with 2D carotid artery MRI can be achieved and may allow for visualization of smaller quantities of IPH or improved quantification. Three-D T1w GRE BB is also compatible with multiple platforms, is easy to interpret, and allows for a large field of view. Use of 3D T1w GRE BB and 3D TOF allows for assessing carotid disease from the aortic arch to the circle of Willis. In contrast, 2D carotid artery MRI acquisitions are usually limited, due to time of acquisition, to a couple of centimeters around the bifurcation. Finally, 3D carotid artery MRI allows for creation of multiplanar 3D reconstructions and improved 3D to 3D registration of studies acquired at different time points, thereby improving longitudinal study.

Given that the trial patients had nonsurgical carotid disease, the reference standard of 2D carotid artery MRI, rather than histology, was used to evaluate agreement of 3D carotid artery MRI measurements. However, 2D carotid artery MRI imaging is generally regarded as the present MRI imaging gold standard. Three-D and 2D carotid artery MRI had excellent agreement, suggesting that both are useful for measuring changes in VW volume. Unlike multicontrast 2D imaging (Yuan et al., 2001), 3D T1w BB imaging did not allow for reliable characterization of the lipid core. Others have, however, suggested that a combination of three 3D carotid artery MRI sequences (time-of-flight (TOF), MP-RAGE, and MERGE) have sufficient contrast to allow for identification of necrotic core, calcium, and loose matrix (Liu et al., 2012). The consideration of plaque components other than IPH was beyond the scope of this study and may warrant further investigation.

5.6 Conclusions

3D carotid artery MRI at 3T, using 3D T1w GRE BB and TOF acquisitions, allows for highly reliable volumetric quantification of VW and IPH. Current clinical imaging trials on 3D carotid artery 3T MRI of IPH may reliably quantify VW and IPH, which may allow for detection of
disease progression. Trials including volumetric quantification of VW and IPH may also allow for improved cerebrovascular risk stratification and insight into the role of carotid IPH in cerebrovascular outcomes. Further studies may also help demonstrate the potential clinical benefits resulting from improvements in quantitative evaluation, image registration, or speed of segmentation.
Chapter 6—General Discussion

This chapter contains sections or content either adapted or reproduced with permission from the following peer-reviewed literature.

6.1 Summary

This thesis investigated magnetic resonance imaging depicted intraplaque hemorrhage (MR-IPH) and its risk factors, presence in patients with embolic stroke of undetermined significance, and relationship with a cardiovascular phenotype. MR-IPH imaging from both a clinical trial and institutional experience was used throughout the thesis. Data herein support the hypotheses that carotid MR-IPH is associated with a high-risk cardiovascular phenotype as indicated by its association with cardiovascular and cerebrovascular risk factors and outcomes.

In this first study (Singh et al., 2017), data from our 10-year institutional experience demonstrated that males have greater age-specific odds of magnetic resonance imaging (MRI) depicted carotid IPH compared to females, and that with increasing age, the odds of carotid IPH in females becomes closer to that of males. Among 906 patients with low-grade carotid stenosis, males were found to have greater odds of carotid IPH than females at ages 45 to 54 (odds ratio (OR)=45.45, 95% confidence interval (CI) 3.43 to 500), 55 to 64 (OR=21.74, 95% CI 3.21 to 142.86), 65 to 74 (OR=10.42, 95% CI 2.91 to 37.04), and ≥ 75 years (OR=5.00, 95% CI 2.31 to 10.75), with odds of IPH converging between the sexes with age. In other words, male sex modified the effect of age on the presence of carotid IPH (β=0.074, SE=0.036, p=0.0411).

In the second study (Singh, Moody, Panzov, & Gladstone, 2018), an embolic stroke of undetermined source sample of patients was used from a clinical trial investigating atrial fibrillation as an etiology. Embolic stroke of undetermined source patients have carotid IPH ipsilateral to the affected brain in a sample of anterior stroke patients without evidence of atrial fibrillation investigated in the clinical trial setting. Ipsilateral and contralateral IPH was found in 7/35 (20.0%) and 3/35 (8.6%) of patients, respectively (p=0.005).

The third study (Singh et al., 2013) found that the odds of a composite cardiovascular (CV) event in the MR-IPH patients was 6.75 (Bi-variable analysis, 95% CI, 2.75 to 16.6, p<0.0001) and 3.25 (Multivariable regression analysis, 1.14 to 9.37, p=0.028). MR-IPH had the highest OR of a prior CV event compared to other variables, including age, sex, hypertension, and stenosis. The OR of individual CV events was also significant: MI (3.35, 95% CI 2.11 to 14.2, p<0.01), coronary stenting (26.4, 95% CI 8.80 to 79.4, p<0.01), coronary angioplasty (21, 95% CI 4.84 to 91.1, p<0.01), and PVD (3.35, 95% CI 1.09 to 10.3, P<0.05).

These studies support the hypothesis that IPH is associated with a high-risk cardiovascular phenotype, including cardiovascular risk factors such as age and sex and both
cerebrovascular and cardiovascular events. The studies raised the question of the significance of IPH volume adjusted for plaque burden for further investigation of in the potential causal relationship between IPH and a cardiovascular and cerebrovascular phenotype. Thus, the fourth study (Singh et al., 2015) developed an approach to quantification and investigated inter-, intra-, and scan-rescan reliability of this 3D carotid artery MRI approach and assessed agreement with 2D T1 MRI. The addition of a 3D T1w gradient echo black blood (GRE BB) acquisition to routine 3D TOF was found to be highly reliable for quantification of VW volume and IPH and also demonstrated excellent agreement with 2D T1 MRI for measuring VW volume. The quantitative imaging analysis protocol is intended for use in carotid imaging trials, including the proposed studies in Chapter 7 that seek to further explore the relationship between carotid MR-IPH and cerebrovascular imaging outcomes.

6.2 Novelty and Strengths

Given that several emerging studies suggest the importance of IPH in plaque progression, future stroke, and myocardial infarction, we exploited the opportunity to provide further insights into IPH pathophysiology, determinants, and outcomes using a large institutional experience and data from a clinical trial. The work in this thesis provides insight into the pathophysiology of IPH, specifically the effect of age and sex on IPH in low-grade stenosis. To our knowledge, no other studies have demonstrated the convergence of risk of carotid IPH between the sexes with increasing age. The work also demonstrates the feasibility of application of IPH imaging in patients undergoing neurovascular imaging to identify patients of a high-risk cardiovascular phenotype. Furthermore, in the case of patients with embolic stroke of undetermined source, IPH imaging is suggested to enhance the diagnosis by identifying culprit lesions as part of the comprehensive search for possible underlying stroke etiology. Reliable quantification of IPH was also demonstrated, and in the context of others’ suggestions that IPH has incremental prognostic value compared to traditional factors alone for risk prediction and net reclassification of cardiovascular outcomes, the ability to quantify IPH is essential in understanding its role in clinical outcomes and establishing clinical cut-offs to ensure optimal thresholds in the trial setting.

Incorporating imaging of IPH into routine clinical practice is feasible. First, the MR pulse sequence required to apply the IPH imaging technique is readily available on all scanner platforms; they are rapidly acquired; IPH detection is accurate and interpretation is simple,
presently comprising dichotomization into presence or absence of IPH. Furthermore, MR imaging at present is the only readily available imaging technique for the detection of plaque hemorrhage, and MR imaging is an already-used technique for investigating carotid artery disease. Thus, the addition of a single sequence to the standard carotid artery imaging protocol is simple with little time-cost impact. Additionally, routinely used neurovascular coils are used; no specialty surface coils are required (Brinjikji et al., 2018). The imaging covers a large field of view from the arch to circle of Willis. Finally, no contrast administration is necessary, and therefore, there is no added risk of nephrogenic systemic fibrosis. Patients with chronic kidney disease are well documented to be at high risk of stroke (Ueda et al., 2011), and silent brain infarction is present in 37.5% of patients with grade three kidney disease (i.e., 30 to 44.9 ml/min/1.73m² clearance) (Chou et al., 2011). Therefore, the absence of contrast administration may be particularly useful when considering that high stroke risk populations such as those with chronic kidney disease may benefit from risk stratification with carotid artery MRI (Rubin, Rosas, Chirinos, & Townsend, 2011).

6.3 Limitations

Where possible, clinical trial data was used in this thesis. However, limitations include the use of retrospective data collection that made it challenging to always access complete records and obtain granular level data. Resource constraints limited the number of steps that could be taken to obtain more granular data such as accessing administrative databases, interviews by telephone or in person, or tapping other networks or administrative databases. While linking with administrative datasets via Institute of Clinical Evaluative Sciences or with registries available through networks such as the Canadian Stroke Network were a few options, infrastructure constraints limited the ability to pursue these avenues over the duration of this thesis.

Nonetheless, the generalizability of this work that reflected data collected in routine clinical practice is one distinct advantage (Patsopoulos, 2011). The biomarker of interest was imaged in the routine clinical setting and therefore informs on its use in practice. Additionally, retrospective databases are relatively time-efficient and inexpensive, allowing for large study populations (Motheral et al., 2003). To prospectively collect information on 2,000 patients in an MRI trial would have been cost-prohibitive, and assuming a cost of $1,500/patient, would require approximately $3M to construct the database used in this thesis. Therefore, this thesis allows assembly of a large database of patients in an efficient way.
6.4 Relationship to Other Work

6.4.1.1 Clinical Application

Addition of IPH imaging to routine carotid artery MRI assessment has the potential to impact patient management in a number of clinical settings including whether the patient is: asymptomatic but at high risk of vascular disease; symptomatic and being assessed for risk of future event; or in the work up prior to carotid artery intervention. Newer application may include identifying high risk disease requiring modified, intensive, or costly therapy; identification of patients requiring increased monitoring; entry criteria for therapeutic trials; and phenotyping of high risk patients who may require more comprehensive vascular investigation and potentially therapy modification.

6.4.1.2 Primary Prevention

In the absence of organized vascular screening programs, asymptomatic carotid arteries are most commonly imaged because of high risk atherosclerotic vascular disease in another vascular bed such as the coronary or peripheral vascular circulation (Brott et al., 2011). Alternatively, asymptomatic vessels will commonly be imaged in conjunction with a symptomatic contralateral carotid artery. Despite the silent nature of these carotid vessels, study of asymptomatic cohorts has repeatedly demonstrated an increased risk of future clinical events irrespective of the degree of stenosis in those patients with MR demonstrated IPH rather than those without (Altaf, MacSweeney, Gladman, & Auer, 2007; Singh et al., 2009). The very low rate of events in the IPH negative groups is probably an even more compelling reason to investigate patients using MR-IPH imaging. The absence of IPH appears to confer a level of low risk that might be useful in managing patients, even those with significant carotid artery stenosis, potentially eliminating the need for invasive interventions (Gupta & Marshall, 2015; Treiman, McNally, Kim, & Parker, 2015). Carotid vessels with no stenosis are also found to contain IPH, suggesting that advanced vessel wall disease indicated by IPH can occur even when vessel wall thickening is minimal. Large cohort trials currently underway may provide information on the relevance of finding this high risk biomarker in otherwise normal people, potentially confirming whether this can act as an early marker of future events even in the absence of other, more established risk markers.
Asymptomatic patients found to have IPH may be candidates for more intensive management despite the lack of recognized risk factors such as luminal stenosis. Patients screened for carotid disease because of vascular disease elsewhere are known to have asymptomatic nonstenotic carotid disease identified with IPH MRI (Cheung et al., 2011). Because IPH is a stimulus for plaque progression (Takaya et al., 2005) and potentially carotid stenosis progression (Singh & Moody, 2015), these patients could be identified for more follow up to better detect the occurrence of significant stenosis (which may then be eligible for surgical intervention). Prior to the need for surgical intervention, the risk benefit of more intensive medical therapy can be weighed because these patients are at higher risk of disease progression.

The concept of identifying the high risk patient by identifying high risk lesions could be further expanded. From the prospective study of clinical outcomes of patients with histologically proven IPH in their carotid endarterectomy specimens (Hellings et al., 2010) and the retrospective review of comorbid cardiovascular conditions in patients with and without carotid IPH (Singh et al., 2013), it appears that the status of the carotid artery, and in particular the presence or absence of IPH, provides a useful biomarker of the patient’s vascular phenotype. IPH in the carotid bed therefore seems to be associated with symptomatic vascular disease peripherally and in the coronary circulation, and it is also able to predict risk of future cardiovascular events. While it could be argued that medical therapy for one vascular bed (i.e., carotid) will have an equally beneficial effect in all vascular beds without the need for further investigation, a case could be made for the investigation and exclusion of significant, silent lesions, for instance, in the proximal coronary arteries, once carotid IPH has been detected.

Knowing that IPH represents a marker of increased risk of atherosclerotic progression and clinical outcomes, the technique could be used to improve the selection of patients for proof of principle trials of new interventions. Using MRI detected IPH as a predictive classifier to select those patients at greater risk of events, smaller recruitment numbers will be required, allowing a more efficient demonstration of effect.

6.4.1.3 Secondary Prevention

Patients with symptoms referable to the carotid artery circulation should always undergo carotid artery investigation. First, defining the cause of a cerebral event impacts future therapy or intervention and impacts accurate diagnosis of carotid artery disease because the source of
thrombo-emboli excludes other sources such as the heart. However, the current diagnostic criteria for diagnosing significant carotid artery disease relies on the identification of a 50% stenosis or more (Adams et al., 1993). Less than this, and the carotid artery is considered asymptomatic. In reality, degrees of stenosis less than 50% can cause cerebral thrombo-emboli, although the incidence of events decreases with the level of stenosis. It should be remembered, however, that the cumulative number of cerebral events in the nonstenotic group is significant. MR-IPH has been shown to be present even in low-grade (<50%) carotid artery stenosis and associated with symptoms. While prospective outcome trials of this biomarker in this "nonstenotic" group are yet to be performed, identification of IPH with MRI in these patients may be useful in identifying symptom-causing carotid artery disease. However, because there is not a direct cause and effect relationship between IPH and cerebral symptoms, further study of the significance of IPH detection in these patients with cerebrovascular symptoms is required.

Commonly, patients are investigated for the causes of stroke, and none, using current criteria, are found—the diagnosis remaining embolic stroke of undetermined source. While a direct causal relationship between IPH and stroke cannot be made, one of the first descriptions of MRI detected IPH (A. R. Moody et al., 1999) was in acute stroke patients, which noted that 5/11 patients with MRI high signal indicative of IPH had carotid stenosis less than 50%. Using MRI to detect IPH may identify culprit lesions associated with stroke which otherwise would be classified as cryptogenic. More recently, similar results were shown in DWI proven stroke patients without significant (>50%) stenosis who demonstrated IPH ipsilateral to the index cerebral infarct in 6/27 patients (22.2%), but none on the contralateral side (Gupta, Gialdini, et al., 2015). As in many areas of carotid artery investigation, imaging for IPH has the potential to add to the knowledge already provided by the current imaging techniques.

Due to the increasing interest in IPH, there are now sufficient studies that allow meta-analysis of multiple trials concerning the relationship of IPH and cerebrovascular symptoms. Studies by Hosseini et al. (OR 12.2) (Hosseini et al., 2013), Saam et al. (HR 5.69) (Saam et al., 2013), and Gupta et al. (HR 4.59) (Gupta et al., 2013) all showed the positive association of symptoms with IPH.

Treiman et al. (Treiman et al., 2015) projected the probability of stroke occurrence, assuming an annual rate of symptomatic stroke of 2.2% and 4.0% of silent cerebral infarction in the setting of a 5.2 hazard ratio for stroke in the presence of IPH. Projections to four years
showed the probability of symptomatic stroke with IPH was 18% and of symptomatic/asymptomatic stroke combined was 43%. This compares with projections of 3% and 8%, respectively, for IPH negative patients.

6.4.1.4 Progression of Vessel Wall Disease

From the pathobiology of IPH, it can be seen that the pro-inflammatory nature of free hemoglobin within the plaque will be a stimulus for plaque growth through a mixture of attempted repair and healing, lipid accumulation, and repeated plaque hemorrhage. Plaque growth initially may be through positive remodeling with no encroachment upon the lumen, but eventually, this will be expressed as an increasing degree of luminal stenosis. The positive remodeling phase measurement of stenosis is therefore a poor means of detecting vessel wall disease. Detection of vessel wall disease when there is no lumen stenosis, by the addition of vessel wall imaging, is therefore particularly important.

IPH has been shown in a number of studies to predict progression of vessel wall disease. More recently, this has been shown to be translated into progression of clinical stenosis as well (Singh & Moody, 2015). The importance of IPH therefore seems amplified because not only may it result in plaque destabilization, potential plaque rupture, and end organ ischemia, but it can also produce a more gradual and persistent effect by driving plaque progression. The latter eventually reaches a stage in which clinical events become manifest. The persistent presence of IPH can therefore be thought of as acting as a slow, continual stimulus for plaque growth, likely leading to further bleeding within the plaque and then punctuated with more sporadic and unpredictable plaque rupture. The latter is likely dependent on local features of the plaque, including geometry, hemodynamics, plaque composition, circulating factors, and vascular function. The presence of IPH during the nonstenotic phase may therefore provide a useful marker of those lesions more prone to increases in plaque volume and thus eventual increased risk of future events. This provides a theoretical opportunity to target patients for more intensive follow-up and medical therapy in an attempt to halt or potentially reverse the vessel wall disease. These trials however have not yet been undertaken using the presence of IPH as the entry criteria for treatment.
6.4.1.5 Pre-intervention Imaging

The relationship of IPH, as demonstrated by MRI, to periprocedural outcomes for carotid interventions has been studied. Overall, as might be expected, the presence of IPH confers an increased risk for the patient undergoing carotid intervention. This is not surprising because the presence of plaque hemorrhage indicates a more advanced and complex morphology, such that any manipulation of the carotid artery may result in thrombo-embolic disease. Even prior to surgery, there is evidence that the resting rate of high intensity signals (HITS) on transcranial Doppler is increased from carotid arteries containing IPH (Altaf et al., 2011). During endarterectomy surgery, manipulation of carotid arteries containing IPH also results in more perioperative HITS (Altaf, Beech, et al., 2007).

Carotid stenting might appear an attractive alternative to avoid the IPH risks associated with carotid endarterectomy. However, the presence of IPH also increases the risks associated with carotid artery stenting, resulting in more periprocedural cerebro-ischemic events compared with endarterectomy (Yoshimura et al., 2011). Because the presence of IPH appears to confer increased risk during carotid interventions, preprocedural imaging may be useful in interventional planning and may provide insight into potential risks on a lesion-by-lesion basis.

6.5 Conclusions

Carotid IPH is an emerging biomarker of a high-risk cardiovascular phenotype, including association with cardiovascular risk factors and determinants, and cerebrovascular and cardiovascular events. The shift from evaluation of the carotid artery lumen to vessel wall visualization for IPH may improve cardiovascular and cerebrovascular risk stratification. Understanding determinants of IPH such as its modulation by sex provides insights into potential avenues for therapeutic targeting. If observations on IPH and its relationship with various vascular outcomes are confirmed in prospective and quantitative trials, IPH imaging is certainly clinically feasible and cost effective, providing an opportunity to alter current patient management strategies for improved patient health outcomes.
Chapter 7—Future Directions

This chapter contains sections or content either adapted or reproduced from an awarded peer-reviewed grant competition and the following peer-reviewed literature.


7.1 Opportunity for Study

Several experimental opportunities exist for future investigation, including extension of 3D magnetic resonance imaging (MRI) vessel wall imaging into other vascular beds (Hoshi et al., 2015; J. Wang et al., 2016), improvement of current methods, evaluation of new or hybrid imaging methods in contrast to existing methods (Vesey, Dweck, & Fayad, 2016), and automated image processing to facilitate image processing (DeMarco & Huston, 2014). The potential for global vascular burden and comprehensive MRI vascular imaging remains to be investigated, although whole body imaging requires automated image processing methods, addressing ethical and practical issues regarding incidental findings, and evaluation of cost-effectiveness. One serial imaging trial in particular specifically addresses 3D intraplaque hemorrhage (IPH) imaging discussed in this thesis, the Canadian Atherosclerosis Imaging Network Project 1 (Tardif et al., 2013), and provides an opportunity to better understand the role of carotid IPH's natural history and imaging outcomes. A baseline study for this project on the Canadian Atherosclerosis Imaging Network Project 1 is proposed here to evaluate the relationship between carotid IPH and neuroimaging outcomes.

7.2 Proposal Motivation

Stroke, cerebral small vessel disease (SVD), and cognitive impairment account for major mortality and morbidity in the developed world (Lloyd-Jones et al., 2009; Mott, Pahigannis, & Koroshetz, 2014; Pantoni, 2010). Early identification of patients at risk for these neurovascular diseases remains a challenge. Carotid artery imaging may provide a window into the cardiovascular and neurovascular health of patients, creating an opportunity for early intervention (Carcaillon et al., 2014). Current clinical guidelines use carotid stenosis to calculate vascular risk such as stroke (Brott et al., 2011). Carotid studies demonstrate the limited use of luminal stenosis as a biomarker of vascular events (A. R. Moody, Bitar, Leung, & Maggisano, 2006). For example, in the European Carotid Surgery Trial (ECST), 43.8% of the 3,018 participants with symptomatic disease had only low-grade (<30%) stenosis (N.A.S.C.E.T. Collaborators, 1991; Gorelick, 1999; Rothwell, Gutnikov, & Warlow, 2003). The five-year rate of any ipsilateral stroke in the North American Symptomatic Carotid Endarterectomy Trial (NASCET) trial was 22.2% in the low-grade (<50%) stenosis group. Furthermore, eight to 83 endarterectomies indicated based on the criteria of luminal stenosis and symptoms are needed to
prevent one stroke. The search for in-vivo imaging of plaque characteristics to improve risk stratification of patients at risk of atherosclerotic vascular events (i.e., identification of the vulnerable vascular patient) is of great interest (Finn, Nakano, Narula, Kolodgie, & Virmani, 2010; Naghavi et al., 2003; Singh et al., 2013).

Carotid IPH, a component of complicated atherosclerosis, is an emerging marker of a high risk neurovascular phenotype (Gupta et al., 2013; Singh et al., 2012). Studies, including ours, suggest carotid IPH may be associated with plaque instability (Altaf, MacSweeney, et al., 2007; Cheung et al., 2011; Gao, Chen, Bao, Jiao, & Ling, 2007), plaque progression (Sun et al., 2012; Takaya et al., 2005), stroke (Beach et al., 1993; Gupta et al., 2013; Selwaness, van den Bouwhuijsen, van Onkelen, et al., 2014; Singh et al., 2009), white-matter hyperintensities (WMHIs) (Altaf et al., 2006), and a cardiovascular phenotype (Hellings et al., 2010; Singh et al., 2013). MRI can reliably, rapidly, and noninvasively detect IPH in routine clinical practice (Bitar et al., 2008; A. Moody, 2012; A. R. Moody et al., 1999; A. R. Moody et al., 2006; A. R. Moody et al., 2003; Murphy et al., 2003). Systematic and quantitative evaluation of carotid IPH and outcomes may facilitate translation of IPH imaging into clinical use to benefit patients (T. Saam et al., 2005; Sun et al., 2014; Underhill, Hatsuikami, Fayad, Fuster, & Yuan, 2010). Trials are thus underway to prospectively evaluate the relationship of carotid IPH and vascular outcomes (Tardif et al., 2013; Turc, Oppenheim, Naggara, Eker, Calvet, Lacour, Crozier, Guegan-Massardier, Henon, Neau, Toussaint, Mas, Meder, & Touze, 2012; Wasserman, Astor, Sharrett, Swingen, & Catellier, 2010; X. Q. Zhao et al., 2014).

Using one such national clinical trial of serial 3D carotid and brain MR imaging at 3T of ~450 participants (Tardif et al., 2013), the Canadian Atherosclerosis Imaging Network (CAIN), we propose to investigate the baseline relationship of quantitative carotid IPH and quantitative neuroimaging features of SVD (Wardlaw et al., 2013), including WMHIs, lacunes, and brain atrophy. Quantification of both carotid IPH and SVD would utilize our established protocols. Secondarily, we aim to assess the relationship of i) carotid IPH and clinical cerebrovascular outcomes and ii) carotid IPH and cognitive impairment.
7.3 Carotid Intraplaque Hemorrhage and Neurovascular Imaging Outcomes

WMHIs are neuroimaging features of SVD that are of particular interest due to their ability to predict stroke, dementia, and death (Debette & Markus, 2010; Fujikawa, Yamawaki, & Touhouda, 1993; Vermeer et al., 2003). Population studies such as the Rotterdam study have associated WMHIs with measures of atherosclerosis (Bots et al., 1993). The exact etiology of WMHIs remains unclear. The leading school of thought is that they are secondary to the occlusion of the small arteries supplying the brain.

Carotid artery disease is a well-known source of thromboembolism to the brain and has been implicated in 20% to 30% of strokes. Studies have, however, failed to establish a convincing link between carotid artery disease measured by stenosis and WMHIs (Schulz, Gruter, Briley, & Rothwell, 2013; Streifler et al., 1995). Failure to identify potentially targetable contributors of WMHIs is a missed opportunity to curb irreversible cerebrovascular outcomes, including dementia and stroke. The failure to link WMHIs and carotid stenosis is likely due to current studies being underpowered for the detection of WMHIs (Kwee et al., 2011), use of insensitive measures to assess WMHIs (e.g., visual scales rather than reliable quantification techniques) (Schulz et al., 2013), and failure to consider atherosclerotic plaque components in the vessel wall (Finn & Narula, 2012; Underhill et al., 2010). Furthermore, distinction of WMHI type, periventricular versus deep, may also be important (Kandiah, Goh, Mak, Marmin, & Ng, 2014).

Plaque instability, increasingly associated with the presence of carotid IPH, may increase the risk of thromboembolism resulting in downstream small vessel brain infarcts. Carotid IPH has been retrospectively associated with increased WMHI volume in a cross-sectional study (Altaf, Morgan, et al., 2008). Two other underpowered exploratory studies are also suggestive of an association (Altaf et al., 2006; Patterson et al., 2009). The only prospective observational study that attempted to assess WMHI related to IPH had a follow-up period of one year in 50 patients (Kwee et al., 2011), which is likely underpowered. Systematic prospective study of quantitative IPH and quantitative WMHIs is lacking. Demonstration of the ability of carotid IPH to contribute to WMHIs potentially elucidates a novel etiology for WMHIs (i.e., unstable carotid plaque with IPH).
7.4 Carotid IPH, Lacunes, and Brain Atrophy

Lacunes, presumed to be of mostly vascular origin due to small subcortical infarcts of perforating arteriole region, are another neuroimaging feature of SVD that is of interest (Wardlaw et al., 2013). Frequently seen in asymptomatic patients on imaging, lacunes are associated with subsequent risk of stroke, gait impairment, cognitive impairment, and dementia (Benjamin et al., 2014; Santos et al., 2009; Wardlaw et al., 2013). The relationship between carotid atherosclerosis and lacunes has been suggested (Brisset et al., 2013; Fanning, Wong, & Fraser, 2014; Hong et al., 2011). Studies have yet to address whether carotid IPH may play a role in the development of lacunes (Fanning et al., 2014). Given the presumed etiology of lacunes being occlusion of perforating arterioles (e.g., subcortical infarcts), unstable carotid plaques are hypothesized to play a role. CAIN patients have proton density (PD) and T1-weighted neurovascular MR acquisitions, allowing for quantification of lacune volume (Wardlaw et al., 2013).

Cerebral atrophy may be an important measure of the effect of vascular disease burden and possibly a reason for changes in cognition due to vascular lesions. Atrophy has also been associated with carotid atherosclerosis, although the specific role of carotid IPH remains to be evaluated (Jochemsen et al., 2014; Muller et al., 2011). Vascular related atrophy may be preventable, and whether identification of patients at risk of atrophy using their carotid IPH status may be useful is of interest. CAIN includes neuroimaging, including T1-weighted acquisitions with good grey-white differentiation at an isotropic resolution <1 mm allowing for quantitative volumetric assessment of atrophy (Wardlaw et al., 2013).

Other neuroimaging features of SVD, including cerebral microbleeds and perivascular spaces, are of interest. Their quantitative protocols are under development.

7.5 Carotid IPH and Clinical Cerebrovascular Outcomes

IPH has been shown to be a marker of future neurovascular ischemic events (Altaf, MacSweeney, et al., 2007; Gao et al., 2007; Gupta et al., 2013; Lindsay et al., 2012; McNally et al., 2012; Pasterkamp & van der Steen, 2012; Singh et al., 2009; Takaya et al., 2006; Turc, Oppenheim, Naggara, Eker, Calvet, Lacour, Crozier, Guegan-Massardier, Henon, Neau, Toussaint, Mas, Meder, Touze, et al., 2012). We previously reported an association between IPH and neurovascular outcomes in a peripheral vascular disease patient population with
asymptomatic carotid disease (Singh et al., 2009). Our findings were in line with those (hazard ratio, 5.2; \( p = .005 \)) reported by Takaya et al. for the relationship between IPH and future ipsilateral cerebrovascular events (Takaya et al., 2006). The relationship is also consistent with the findings of Altaf et al., who concluded that the presence of carotid MR-IPH predicts the recurrence of ipsilateral cerebrovascular events in symptomatic patients with high-grade stenosis (Altaf, MacSweeney, et al., 2007) and moderate stenosis (Altaf, Daniels, et al., 2008). Although the relationship between carotid IPH and stroke has been reported in various groups (Singh et al., 2012), results from CAIN may further contribute to the body of evidence for the role of IPH imaging in clinical practice.

7.6 Carotid IPH and Cognitive Impairment

Carotid stenosis has been associated with decreased scores on the Montreal Cognitive Assessment (MoCA) and Mini-Mental Status Exam (MMSE) (Buratti et al., 2014; Guo et al., 2014; Kirkpatrick, Vincent, Guthery, & Prodan, 2014; J. Watanabe et al., 2014). Improved scores have been reported subsequent to endarterectomy, suggesting that carotids may be associated with altered cognition (J. Watanabe et al., 2014). Whether the presence of carotid IPH is associated with decreased scores remains to be studied. Curbing cognitive impairment by targeting high risk vascular patients phenotyped via their carotids remains to be evaluated.

7.6.1 Novelty of the Proposed Study

Using a national clinical trial of serial 3D carotid and brain MR imaging at 3T of ~450 participants (Tardif et al., 2013), we propose to investigate the relationship of quantitative carotid IPH and quantitative neuroimaging features of SVD. Prior studies that have evaluated the relationship of carotid IPH and SVD have only considered WMHIs. The WMHI studies are limited because they are generally conducted using low resolution imaging, retrospective designs, small sample sizes, varying imaging techniques that are less clinically useful (i.e., have longer scan times), or are restricted to small groups of specific patient types. The single prospective IPH and WMHI study is underpowered. Potential carotid IPH outcomes, including lacunes, cerebral atrophy, and cognitive impairment measures, remain to be studied. In our study, quantification of both carotid IPH and SVD will utilize our established protocols. We also propose to study the relationship between carotid IPH and SVD features prospectively, with a high power (\( \beta \geq 0.9 \)), and using reliable quantitative methods, including a histologically
validated 3D MRI technique to quantify IPH (Bitar et al., 2008). The use of the 3D MRI technique improves the accuracy of quantification due to its ability to image isotropic voxels. The technique is easily implementable from a technical perspective (e.g., use of MRI protocols, ease of radiologist interpretation), and we have published prior studies from data collected in routine clinical practice (Cheung et al., 2011; Singh et al., 2009; Singh et al., 2013).

7.6.2 Clinical Implications

Improved risk stratification of carotid atherosclerosis patients may provide an opportunity for early intervention, decreased mortality, and societal cost-benefits (Finn et al., 2010; Finn & Narula, 2012; Fleg et al., 2012; Hatsukami & Yuan, 2010; Naghavi et al., 2003; Virmani, Kolodgie, Burke, et al., 2005). Understanding the role of carotid IPH as an etiology of WMHIs and other SVD correlates may be one avenue to target patients. Questions remain about whether patients with carotid IPH may benefit from clinical surveillance or altered clinical management, including the treatment of their unstable atheroma. Large scale trials are emerging, and CAIN provides an opportunity to contribute to the growing literature on carotid IPH by uniquely reporting neuroimaging outcomes in a prospective setting with a large number of patients for an MRI trial. While further trials may be required, the proposed study aims to facilitate the translation of IPH imaging into clinical practice.

7.6.3 Preliminary Studies

Several studies conducted in our carotid imaging laboratory form the basis for the current proposed study (Bitar et al., 2008; Cheung et al., 2011; A. Moody, 2012; A. R. Moody et al., 1999; A. R. Moody et al., 2006; A. R. Moody et al., 2003; Murphy et al., 2003; J. Ramirez et al., 2018; Sarkar et al., 2009; Singh et al., 2009; Singh et al., 2013; Singh et al., 2012; Tardif et al., 2013). With regard to the quantitative evaluation of the 3D carotid 3T MRI, we reported that 3D carotid artery 3TMRI is highly reliable for the volumetric quantitative evaluation of IPH ($ICC_{intra}=.998$, $ICC_{inter}=.996$, $ICC_{scan-rescan}=.949$) (N. Singh et al., 2015). Reproducibility of quantification of neuroimaging features of SVD, specifically WMHIs, lacunes, and brain atrophy, have also been established by our collaborating group (Dade et al., 2004; McNeely et al., 2015; J. Ramirez et al., 2015; J. Ramirez et al., 2011; J. Ramirez, McNeely, Scott, Stuss, & Black, 2014; J. Ramirez, Scott, & Black, 2013; J. Ramirez, Scott, et al., 2014). While cerebral
microbleeds and perivascular spaces are also neuroimaging features of interest indicating SVD, their protocols are in development and are excluded from this proposal.

7.7 Research Design and Methods

7.7.1 Overview and Study Design

The proposed study uses baseline data from a multicenter study of participants undergoing serial imaging over two years as part of CAIN (Tardif et al., 2013). Patients undergo imaging and clinical visits at baseline, one year, and two years. Baseline clinical evaluation includes clinical assessment, including a comprehensive history and physical exam, a battery of neurocognitive tests, detailed cardiovascular and cerebrovascular history, medication history, and basic blood work. Baseline imaging evaluation includes MRI of the carotids and brain, including MRI pulse sequences that allow for the assessment and quantification of IPH, and neuroimaging features of SVD, including WMHIs.

7.7.2 Population

Patients are recruited from eight academic tertiary care centers across Canada between March 2010 and March 2015. Study patients are those with nonsurgical carotid disease indicated by stenosis in at least one carotid between 30% to 95%. The primary analysis group will be those with no carotid intervention prior to or during the course of the study. Patients with >70% stenosis in either carotid artery or carotid intervention during the follow up will be treated as a separate group in the analyses for the proposed study. Patients with >70% stenosis are indicated for carotid endarterectomy based on NASCET (N.A.S.C.E.T. Collaborators, 1991) and ESCT (Rothwell et al., 2003) are more likely to undergo carotid intervention and are therefore considered a separate group to minimize selection biases. Exclusion criteria include i) contraindication for MRI including a GFR<30ml/min that may increase risk of contrast-related nephrogenic cystic fibrosis (Daftari Besheli, Aran, Shaqdan, Kay, & Abujudeh, 2014) or ii) patients with non-MRI compatible implants or hardware (Shellock & Spinazzi, 2008).

7.7.3 Image Acquisitions

Three-D carotid MRI acquisitions include 3D MR-IPH and 3D TOF imaging. Three-D MR-IPH is performed using a noncardiac gated T1-weighted fat-suppressed (using a selected water excitation RF pulse) fast field echo sequence, with imaging acquired in the coronal plane
(repetition time (TR), 11 msec; echo time (TE), 4 msec; field of view (FOV), 270x190 mm²; matrix, 512x256 mm²; slices, 100; through-plane thickness, 0.5; and voxel size 0.5 mm x 0.7 mm x 0.5 mm). Shimming is prescribed for a 10 cm region centered over the neck to ensure B₀-field homogeneity around the carotid arteries. The field of view of 3D MRIPH provides coverage from the top of the aortic arch to the Circle of Willis. Three-D TOF imaging was acquired in the axial plane (TR, 26 msec; TE, 3.5 msec; field of view, 190x190 mm²; matrix, 360x232 mm²; slices, 160 slices; through-plane thickness, 0.7 mm; and voxel size 0.5 mm x 0.8 mm x 0.7 mm). Scan times for 3D MRIPH and 3D TOF are 8:54 and 5:59 minutes, respectively. Neuroimaging acquisitions conform with the National Institute of Neurological Disorders and Stroke vascular cognitive impairment guidelines, and include 3D T1, proton density, FLAIR, T2, and DWI to allow quantitative tissue compartment classification for volumes of SVD.

7.7.4 Image Analysis: Quantification of IPH and Neuroimaging Features of SVD

IPH is quantified using semi-automated segmentation methods using imaging processing software (VesselMass software, Leiden) (van ‘t Klooster, de Koning, et al., 2012; van ‘t Klooster, Naggara, et al., 2012; van ‘t Klooster, Patterson, et al., 2013; van ‘t Klooster, Staring, et al., 2013; van ‘t Klooster et al., 2014). The intra-class correlation coefficients (ICC) for intra-rater reliability, inter-rater reliability, and scan-rescan reliability has been demonstrated to be excellent for the quantification of IPH volume (N. Singh et al., 2015). The details of the re-slicing, registration, and segmentation are also found in our recent work. For IPH quantification, on the axial MRIPH images, the primary slice distal to the flow divider is identified. A region of interest (ROI) was identified on the sternocleidomastoid (ROI area, 20 ± 5 mm²) at the same level as the carotid artery in the direction of frequency encodes (anterior-posterior) to determine signal intensity. A signal intensity of 1.5 times the ROI is used as a threshold to identify IPH in the vessel wall. Classifying presence of IPH by detecting signal intensity greater than 1.5x intensity of the sternocleidomastoid is accepted to determine the presence of IPH (Altatf, Morgan, et al., 2008; Hosseini et al., 2013; Singh et al., 2009; Singh et al., 2013). A ROI is drawn around the outer VW boundary, and a signal above the threshold is included within the boundary. The reviewer is blinded to clinical status and the volume of WMHI disease.

WMHI will be quantified by a second and independent neuroimaging processing laboratory with an established protocol for WMHI analysis. The imaging laboratory uses
experienced and trained imaging analysts, as well as semi-automated registration, segmentation, and quantification techniques using Lesion-explorer software (McNeely et al., 2015; J. Ramirez et al., 2015; J. Ramirez et al., 2011; J. Ramirez, McNeely, et al., 2014; J. Ramirez et al., 2013; J. Ramirez, Scott, et al., 2014). Lesion explorer exploits proton density MRI, as opposed to fluid-attenuated inversion recovery (FLAIR) MRI, to prevent underestimation of WMHIs. Regional quantification of disease provides information, including by hemisphere. Therefore, WMHI volume ipsilateral to each carotid artery can be used as an outcome. Secondary analyses may consider the type of WMHI (e.g., deep or periventricular) because others have suggested separate etiological mechanisms (Kandiah et al., 2014). The protocol has been used in other studies and has demonstrated reproducibility. The neuroimaging laboratory and analysts are blinded to carotid artery disease status and clinical patient status.

7.7.5 Statistical Analysis

Statistical analysis will be performed by the author of this thesis and by an independent biostatistician using statistical software (e.g., SAS Version 9.2, SAS Institute, Cary, NC, USA). A p-value of less than 0.05 will indicate a significant difference. Descriptive statistics will be calculated for all variables of interest. Continuous variables with normal distributions will be reported with mean and standard deviations. Nonnormal distributions will have median and inter-quartile range reported. Categorical variables will be reported as proportions. Normality will be assessed by the Shapiro-Wilk test. Patients with and without carotid IPH will be compared on categorical baseline variables using chi-square and continuous baseline variables using two sample two-sided t-tests (or Wilcoxin Rank Sum test in the event of nonnormal outcomes). Multivariable regression analyses, adjusted for risk factors significantly different between the IPH groups, will be performed to evaluate whether IPH is an independent risk factor or predictor of outcomes of interest (e.g., SVD, cerebrovascular outcomes, cognitive impairment).

With regard to the primary aim, we propose to model carotid IPH volume’s relationship with ipsilateral WMHI volume with a linear regression model: $$E(Y) = B_0 + B_1I(L=1) + B_2(X)$$. In the model, WMHI volume is indicated by Y, IPH volume is indicated by X, and L indicates carotid side. The model will be adjusted for confounders, including age, sex, hypertension, carotid stenosis, and presence of symptoms at baseline. Collinearity will be tested using variance inflation factor analysis, and collinear covariates will be excluded from the model. The model
will be tested using standard methods, including tests for parallelism and appropriateness of the assumption of linearity by comparison with analysis of response profiles.

7.7.6 Strengths and Limitations

Methodological strengths of this proposal include the prospective multicenter trial design, high power to investigate the relationship between IPH and SVD (and secondary outcomes), and use of semi-automated quantification methods that have been demonstrated to be reliable. Methodological limitations include loss of a small number of patients due to inability to complete baseline MRI or analyze their acquisitions with a failure rate of <5 to 7%.

7.8 Future of Cardiovascular Imaging

Noninvasive cardiovascular imaging technology and applications are rapidly emerging. The last two decades have “witnessed an explosive expansion in the armamentarium of noninvasive…imaging technologies capable of providing detailed information about the structure and function of the heart and vasculature” (Di Carli, Geva, & Davidoff, 2016). Noninvasive cardiovascular imaging provides an avenue to phenotype patients in the clinical and research settings for identifying patients with vessel wall atherosclerosis, quantifying its burden, and examining involvement of various end organs. From a biomedical research perspective, insights might be gained into the pathophysiology of disease. As discussed, various authors have studied IPH using MRI and have provided insights, including risk factors for IPH, potentially allowing for targeted and personalized intervention. Understanding the role of IPH in end-organ outcomes provides impetus to pursue development of therapies targeting IPH.

Advancing technologies result in the sequelae of clinical utilization and increasing costs to the health-care system. This necessitates responsible use of advanced cardiovascular imaging techniques. The future of cardiovascular imaging then involves not only development of imaging technologies and using them to gain insights into the pathophysiology of disease processes, but also ensuring that they are truly patient-oriented and effective in the clinical setting. What then makes noninvasive cardiovascular imaging patient-oriented and effective? In the early 1990s, Fryback and Thornbury described the hierarchal levels of evidence of diagnostic tests: i) technical quality—is the test reliable, valid, and accessible?; ii) diagnostic accuracy—is it better than an alternative or has a better cost-benefit ratio?; iii) impact—does it impact clinical decision-making, and how does it compare to other tests with regard to impact on clinical
decision-making?; iv) therapeutic impact—does it improve selection of patients who may benefit from a given therapy?; and v) patient and societal outcomes—does the test improve outcomes cost-effectively? In addition to discussing the role of carotid MRI for studying pathophysiology of disease, this thesis discussed many of these patient-oriented aspects of carotid MRI and MR-IPH imaging effectiveness, and the balance of evidence seems to point to carotid MR-IPH being a patient-oriented and cost-effective test.

Finally, the responsible use and logistics of noninvasive cardiovascular technologies will surely require interdisciplinary collaboration while attempting to maximally improve patient outcomes. Medical imaging specialists in particular may play a role in identifying the best tests to answer clinical questions, ensure validity of techniques being used, develop quantitative protocols, and extract information on the pathophysiology of disease processes. Imaging specialists are also well positioned to maximize research efficiencies and make the most of imaging through the development of quantitative translational research programs that might serve a dual clinical and research purpose.


Finn, A. V., & Narula, J. (2012). Intraplaque hemorrhage: most dangerous is the wound that bleedeth inwardly. JACC Cardiovasc Imaging, 5(8), 856-858.


