SUBCORTICAL HYPERINTENSITIES IN ALZHEIMER’S DISEASE AND THE ELDERLY:
An MRI-based study examining signs of cerebrovascular disease and dementia.

by

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A thesis submitted in conformity with the requirements for the degree of
Doctor of Philosophy
Graduate Department of Institute of Medical Science
University of Toronto

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Institute of Medical Science, University of Toronto

Abstract

Subcortical hyperintensities (SH) are believed to be observable signs of cerebrovascular disease, indicating some form of subcortical vasculopathy. Also commonly referred to as leukoariosis, these hyperintense signals on proton density, T2-weighted and fluid attenuated inversion recovery magnetic resonance images, are commonly observed phenomena in Alzheimer’s disease patients and elderly persons. Several SH sub-types with differential brain-behavior associations have been proposed in the scientific literature: periventricular, deep white, cystic fluid filled lacunar-like infarcts and perivascular Virchow-Robin spaces. This study will present Lesion Explorer (LE): a comprehensive tri-feature MRI-based processing pipeline that effectively and reliably quantifies SH sub-types in the context of additional brain tissues volumetrics in a regionalized manner. The LE pipeline was validated using a scan-rescan procedure. Finally, the LE pipeline was applied in a cross-sectional study of Alzheimer’s disease patients and normal elderly controls. Brain-behavior relationships were demonstrated with regional SH volumes and executive functioning, speed of mental processing, and verbal memory.
Acknowledgments

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Contributions

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List of Abbreviations

Abbreviations
3D  =  Three-dimensional
Aβ42 = amyloid β_{1-42}
AD  = Alzheimer's Disease
ADC  = apparent diffusion coefficient
ADNI  = Alzheimer's Disease Neuroimaging Initiative
AIR5  = Automated Image Registration v.5.2
ApoE  = apolipoprotein E
ARWMC  = Age-Related White Matter Changes
ASL  = arterial spin labelling
BET  = Brain Extraction Tool
BOLD  = blood oxygen level dependent
BPF  = Brain parenchymal fraction
cc  = cubic centimeters
CADASIL  = Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CHIPS  = Cholinergic HyperIntensities Scale
CP  = choroid plexus
CSF  = cerebrospinal fluid
CT  = Computed tomography
CVD  = Cerebrovascular disease
DLB  = Dementia with Lewy Bodies
DSM  = Diagnostic and Statistical Manual of Mental Disorders
dwSH  = deep subcortical hyperintensities
FCM  = fuzzy C-Means
FDG  = fluorodeoxyglucose
FLAIR  = fluid-attenuated inversion recovery
fMRI  = functional magnetic resonance imaging
FMRIB  = Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FSL  = FMRIB Software Library
FTD  = frontotemporal dementia
GM  = gray matter
HfB  = head-from-brain
ICC  = intra-class correlation coefficient of reliability
ITK  = Insight ToolKit
kNN  = k-nearest neighbour algorithm
LabVol = Lesion Explorer segmentation
LE  = Lesion Explorer
MoCA  = Montreal Cognitive Assessment tool
MMSE  = Mini-Mental State Examination
MRI  = magnetic resonance imaging
NAWM  = normal appearing white matter
NC  = normal control
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NIA</td>
<td>National Institute on Aging</td>
</tr>
<tr>
<td>NINCDS</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke</td>
</tr>
<tr>
<td>NPI</td>
<td>Neuropsychiatric Inventory</td>
</tr>
<tr>
<td>ADRDA</td>
<td>Alzheimer's Disease and Related Disorders Association</td>
</tr>
<tr>
<td>PD</td>
<td>proton density</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PPA</td>
<td>primary progressive aphasia</td>
</tr>
<tr>
<td>PROSPER</td>
<td>PROspective Study of Pravastatin in the Elderly at Risk</td>
</tr>
<tr>
<td>pvSH</td>
<td>periventricular subcortical hyperintensities</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SABRE</td>
<td>Semi-Automated Brain Region Extraction</td>
</tr>
<tr>
<td>sCSF</td>
<td>sulcal cerebrospinal fluid</td>
</tr>
<tr>
<td>SH</td>
<td>subcortical hyperintensities</td>
</tr>
<tr>
<td>SI</td>
<td>Similarity index</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computed tomography</td>
</tr>
<tr>
<td>SPGR</td>
<td>Spoiled gradient recalled</td>
</tr>
<tr>
<td>T1seg</td>
<td>T1-based segmentation</td>
</tr>
<tr>
<td>T2</td>
<td>T2-weighted</td>
</tr>
<tr>
<td>TIC</td>
<td>Total intracranial capacity</td>
</tr>
<tr>
<td>ST-TIC</td>
<td>Supra-tentorial intracranial capacity</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>VBM</td>
<td>voxel-based morphometry</td>
</tr>
<tr>
<td>VCI</td>
<td>Vascular cognitive impairment</td>
</tr>
<tr>
<td>vCSF</td>
<td>ventricular cerebrospinal fluid</td>
</tr>
<tr>
<td>VR</td>
<td>Virchow-Robin spaces</td>
</tr>
<tr>
<td>WM</td>
<td>white matter</td>
</tr>
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**SABRE Regions**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Region Description</th>
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<tbody>
<tr>
<td>LSUPF</td>
<td>Left Superior Frontal</td>
</tr>
<tr>
<td>LIF</td>
<td>Left Inferior Frontal</td>
</tr>
<tr>
<td>LOBF</td>
<td>Left Orbital Frontal</td>
</tr>
<tr>
<td>LMOBF</td>
<td>Left Medial Orbital Frontal</td>
</tr>
<tr>
<td>LSP</td>
<td>Left Superior Parietal</td>
</tr>
<tr>
<td>LIP</td>
<td>Left Inferior Parietal</td>
</tr>
<tr>
<td>LO</td>
<td>Left Occipital</td>
</tr>
<tr>
<td>LAT</td>
<td>Left Anterior Temporal</td>
</tr>
<tr>
<td>LPT</td>
<td>Left Posterior Temporal</td>
</tr>
<tr>
<td>LABGT</td>
<td>Left Anterior Basal Ganglia and Thalamus</td>
</tr>
<tr>
<td>LPBGT</td>
<td>Left Posterior Basal Ganglia and Thalamus</td>
</tr>
<tr>
<td>LMSF</td>
<td>Left Medial Superior Frontal</td>
</tr>
<tr>
<td>LMIF</td>
<td>Left Medial Inferior Frontal</td>
</tr>
<tr>
<td>RSUPF</td>
<td>Right Superior Frontal</td>
</tr>
<tr>
<td>RIF</td>
<td>Right Inferior Frontal</td>
</tr>
<tr>
<td>ROBF</td>
<td>Right Orbital Frontal</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>RMOBF</td>
<td>Right Medial Orbital Frontal</td>
</tr>
<tr>
<td>RSP</td>
<td>Right Superior Parietal</td>
</tr>
<tr>
<td>RIP</td>
<td>Right Inferior Parietal</td>
</tr>
<tr>
<td>ROBF</td>
<td>Right Orbital Frontal</td>
</tr>
<tr>
<td>RAT</td>
<td>Right Anterior Temporal</td>
</tr>
<tr>
<td>RPT</td>
<td>Right Posterior Temporal</td>
</tr>
<tr>
<td>RABGT</td>
<td>Right Anterior Basal Ganglia and Thalamus</td>
</tr>
<tr>
<td>RPBGT</td>
<td>Right Posterior Basal Ganglia and Thalamus</td>
</tr>
<tr>
<td>RMSF</td>
<td>Right Medial Superior Frontal</td>
</tr>
<tr>
<td>RMIF</td>
<td>Right Medial Inferior Frontal</td>
</tr>
</tbody>
</table>
Chapter 1: Thesis Introduction

Based on published work:

1.1 Dementia

1.1.1 Prevalence

Dementia originally comes from Latin terms, meaning “without mind” (\textit{de-} “without”, \textit{mens} “mind”), and can be defined as a deterioration in memory combined with an impairment of one or more areas of cognitive functioning. According to the Canadian Study of Health and Aging, dementia affects 8% of Canadians over age 65, rising to 33% by age 85, with an estimated incidence rate of 103,700 new cases of dementia per year in 2008, and prevalence of over 480,600 Canadians (Alzheimer Society of Canada, 2010). In 2008, the total direct cost of dementia care in Canada was estimated at $15 billion (Alzheimer Society of Canada, 2010). In the US, the prevalence is estimated to be 5.5 million, with an annual cost of $100 billion (Hebert, L. E., Scherr, P. A., Bienias, J. L., Bennett, D. A., & Evans, D. A., 2003). By 2012, cost estimates for health care, long term care, and hospice services required for people over 65 yrs with dementia are expected to be at $200 billion in the US alone (Alzheimer's Association, 2012). In 2005, the prevalence of dementia worldwide was estimated at 29.3 million cases with an estimated cost to society of $315.4 billion USD (Wimo, A., Winblad, B., & Jonsson, L., 2007).

Based on results from the Framingham Study, a prospective study conducted in the US following 4897 participants who were free from strokes and dementia at 55 yrs old, the lifetime risk of stroke or dementia was estimated to be greater than 1 in 3 (Seshadri, S. & Wolf, P. A., 2007). In the absence of new prevention or disease-modifying treatments, given the aging of the human population, dementia constitutes a major challenge for health care and social systems around the world.
Interestingly, results from a recent report by the Rotterdam Study (Larson, E. B. & Langa, K. M., 2012; Schrijvers, E. M. et al., 2012), as well as similar reports from population-based studies in the US (Langa, K. M. et al., 2008; Manton, K. C., Gu, X. L., & Ukraintseva, S. V., 2005), suggest a potential decline in dementia prevalence and mortality rates. The Rotterdam Study examined participants beginning in 1990 and ending in 2005, demonstrating an overall (but statistically non-significant) decline in age-adjusted incidence of dementia, as well as lower mortality rates in the 2000 cohort. It was also noted that hypertension and obesity significantly increased between 1990 and 2000, which was subsequently followed by a strong increase in anti-thrombotics and lipid-lowering drugs. These results suggest that management of vascular risk factors may have contributed to a potential decline in dementia incidence. Additional support for this comes from the Rotterdam Study’s neuroimaging analyses, which found that participants in the more recent sub-cohort (2005-2006) had significantly greater total brain volumes and less evidence of small vessel disease than participants in the earlier sub-cohort (1995-1996), suggesting a decrease in brain atrophy and less evidence of cerebrovascular injury.

The decline in dementia incidence, in both the Dutch and US studies, was attributed to increased wealth, education, and more importantly, improved management of vascular risk factors (Larson, E. B. & Langa, K. M., 2012; Schrijvers, E. M. et al., 2012). It is important to note that with the exception of the neuroimaging results, the Rotterdam Study’s incidence findings were statistically non-significant. However, they do suggest the potential for positive results from health care initiatives that target
education and control of cerebrovascular risk, which may potentially prolong dementia-free life in the elderly.

Certainly cerebrovascular pathology and its contribution to the expression of dementia is gaining attention as a target for research, particularly when combined as a mixed pathology with neurodegenerative disorders such as Alzheimer’s disease (AD) (Black, S. E., Gao, F. Q., & Bilbao, J., 2009; Knopman, D. S., 2012). As the majority of dementia cases are mixed pathologies with a vascular component (Schneider, J. A., Arvanitakis, Z., Bang, W., & Bennett, D. A., 2007), research which seeks to understand the role of vascular pathology remains a priority in current studies on aging and dementia (Deramecourt, V. et al., 2012; Gorelick, P. B. et al., 2011).

In line with the current research, this thesis examines the relationship between AD, cerebrovascular disease (CVD), aging and dementia, through the development and application of complex neuroimaging analysis techniques, which comprehensively and reliably quantify brain tissue and cerebrovascular injury in AD patients and normal elderly. The subsequent sections of this introduction will briefly discuss dementia as a clinical entity, review some of the basic concepts of AD and CVD, followed by an in-depth discussion of how magnetic resonance imaging (MRI) techniques can be used to quantify brain tissue atrophy and subcortical hyperintensities (SH) as surrogate markers of small vessel disease.

1.1.2 Consensus-based characterization & practice parameter

As outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994), dementia is characterized by an acquired and persistent compromise in memory and one other cognitive domain that is
sufficiently severe enough to cause impairment in daily occupational or social functioning. Dementia disorders include a wide and varied group of diseases which often overlap in clinical presentation and patterns of progression. They are often heterogeneous and overlapping, making differential diagnosis difficult, particularly in the context of normal aging.

To assist in differential diagnosis, a number of consensus criteria have been developed to guide recruitment into clinical studies and also aid clinicians to diagnose and manage the care of patients with these disorders. Thus, consensus-based diagnostic criteria have been developed and recently updated to include clinical profiles for: Alzheimer's disease (AD) (McKhann, G. M. et al., 2011), preclinical stages of AD (Sperling, R. A. et al., 2011), mild cognitive impairment (MCI) (Albert, M. S. et al., 2011), frontotemporal dementia (FTD) (Rascovsky, K. et al., 2011), dementia with Lewy bodies (DLB) (McKeith, I. G. et al., 2005), primary progressive aphasia (PPA) (Gorno-Tempini, M. L. et al., 2011), and vascular cognitive impairment (VCI) (Gorelick, P. B. et al., 2011).

In response to the growing complexity and prevalence of these diseases affecting our aging population, the Quality Standards Subcommittee of the American Academy of Neurology (AAN) published an evidence-based practice parameter to provide recommendation guidelines and criteria for the diagnosis, management, and treatment of dementias in the elderly (Knopman, D. S. et al., 2001). This practice parameter included recommendations for the use of the DSM definition for dementia, as well as the National Institute of Neurologic, Communicative Disorders and Stroke – AD and Related Disorders Association (NINCDS-ADRDA) diagnostic criteria for AD.
The AAN practice parameter also recommended the use of structural neuroimaging, such as non-contrast computed tomography (CT) or MRI, in the routine initial evaluation of patients with dementia, reporting greater reliability over functional neuroimaging, genotyping, and other biomarkers. Additionally, the report recommends routine practice assessment and screening for depression, B₁₂ deficiency, and hypothyroidism, all of which are common and treatable comorbidities in the elderly.

In a paired evidence-based report, AAN published a practice parameter regarding the early detection of dementia, which provided useful screening options and clinical monitoring guidelines for individuals with mild cognitive impairment (MCI) (Petersen, R. C. et al., 2001). As there are currently no gold standard consensus criteria regarding an appropriate definition and sub-classification variants (e.g. amnestic...
vs. non-amnestic), MCI as a diagnostic entity remains controversial, with varying definitions and cut-off scores being used across studies (Ganguli, M. et al., 2011).

The criteria for AD and MCI have recently been updated, de-emphasizing memory loss as a *sine qua non* for diagnosis of AD though this is still the prototypic early sign, and incorporated biomarkers such as presenilin mutations or positive amyloid PET into the diagnostic process (Albert, M. S. et al., 2011; McKhann, G. M. et al., 2011). Impairment in two cognitive domains sufficient to impair functional autonomy is now the requirement not only for these new criteria but also for the proposed new DSM-V criteria, which will avoid stigmatizing terms such as “dementia” and replace it with “Major Neurocognitive Disorder” ([www.dsm5.org](http://www.dsm5.org)). An entire issue of *Alzheimer’s and Dementia: The Journal of the Alzheimer’s Association*, was devoted to topics in dementia diagnosis and care, which included amendments to the criteria recommended by the National Institute on Aging (NIA) – Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease (Khachaturian, Z. S., 2011).

1.1.3 **Clinical assessment of dementia**

Clinical assessment of dementia involves a comprehensive, multi-faceted evaluation that combines cognitive, neuropsychiatric, laboratory, and neuroimaging assessments, which will be discussed briefly in the following sections. Additionally, characterization typically includes historical and demographic information when available (eg. age, education, occupation, etc.), which are believed to modulate the expression of dementia. As the dementias are generally characterized as degenerative disorders, longitudinal studies with serial assessments are applied to properly evaluate the gradual progression of the disease through time.
1.1.4 Cognitive assessment

The most widely used cognitive screening instrument is the Mini-Mental State Examination (MMSE), which is a 30-point questionnaire that assesses cognition in 5 subtest areas: Orientation, Registration, Recall, Attention/Concentration/Calculation, and Language (Folstein, M. F., Folstein, S. E., & McHugh, P. R., 1975). Scores obtained from the MMSE are typically summed to obtain a score out of 30, where scores between 21-24 (or 26) may indicate mild cognitive impairment, 10-20 indicate moderate impairment, and scores less than 9 can indicate severe impairment. Recent criticisms regarding the sensitivity, specificity, and predictive value of the MMSE, particularly in regards to its utility in identifying mild cognitive impairments (Lonie, J. A., Tierney, K. M., & Ebmeier, K. P., 2009; Mitchell, A. J., 2009), and reliability for assessing and monitoring treatments (Ballard, C., Corbett, A., & Sharp, S., 2011; Bowie, P., Branton, T., & Holmes, J., 1999), have led to the development of the Montreal Cognitive Assessment tool (MoCA) (Nasreddine, Z. S. et al., 2005).

Similar to the MMSE, the MoCA is scored out of a possible total score of 30 (higher scores indicate better cognitive performance), assessing 6 cognitive domains: Executive Functioning, Visuospatial Abilities, Short-term Memory, Language, Attention, Concentration, and Working Memory (Nasreddine, Z. S. et al., 2005). This freely available test is gaining worldwide popularity as an effective screening tool (partially due to recent enforcement of charges regarding the use of the MMSE-2 as a registered and proprietary screening tool owned by Psychological Assessment Resources incorporated) that has been translated and validated for use in several different languages (Freitas, S., Simoes, M. R., Alves, L., & Santana, I., 2011; Karunaratne, S.,
The MMSE was created prior to the recognition of MCI as a generally accepted clinical state, so the recent development of the MoCA as a brief cognitive screening tool has improved sensitivity for detection of MCI from 18% (MMSE) to 90% (MoCA), as reported in the original publication (Nasreddine, Z. S. et al., 2005). Subsequent comparisons of the MMSE and MoCA report similar improvements with sensitivity and specificity, demonstrating MoCA’s ability to detect cognitive impairment in elderly individuals who achieved adequate MMSE scores in the normal range (Freitas, S., Simoes, M. R., Alves, L., & Santana, I., 2011; Lonie, J. A., Tierney, K. M., & Ebmeier, K. P., 2009; Markwick, A., Zamboni, G., & de Jager, C. A., 2012).

As validation studies worldwide continue to report data regarding the utility of various cognitive screening tools, our understanding of dementia and the possibility of properly assessing the transitional state of MCI as a precursor to dementia, may assist in diminishing the burden of cognitive aging placed on our global health care systems.

1.1.5 Neuropsychiatric assessment

Although structured interview tools such as the Neuropsychiatric Inventory (NPI and its modified brief questionnaire form, NPI-Q) can be used to assess common neuropsychiatric manifestations of dementia and the overall impact on caregivers associated with behavioural changes for whom they care for (Kaufer, D. I. et al., 1998; Kaufer, D. I. et al., 2000), it is also equally important to recognize the insight derived from informal behavioural assessments provided by experienced clinicians. Social behaviour, mood states and other neuropsychiatric symptoms, such as impulsiveness,
passivity, disinhibition, agitation, anxiety, irritability, apathy and purposeless motor
behaviours, can be gauged by a clinician in an informal interview setting and may assist
in differential diagnosis.

1.1.6 Laboratory testing

Although beyond the scope of this thesis, it is important to note that laboratory
tests, other than B₁₂ deficiency and hypothyroidism, are not within the recommended
screening tools outlined in the AAN guidelines for diagnosis of dementia due to
insufficient data regarding their predictive value (Knopman, D. S. et al., 2001).

Regardless, various studies have used genetic testing for apolipoprotein E
(ApoE). Results from these studies have demonstrated that carriers of the ApoE
epsilon 4 (є4) allele have a genetic risk factor for sporadic AD, are more likely to have
increased amyloid burden, have a greater risk for accelerated brain atrophy, and may
respond differentially to particular drug treatments (Bizzarro, A. et al., 2005; Blesa, R. et
al., 1996; Nagy, Z. et al., 1995; Okonkwo, O. C. et al., 2010; Schmechel, D. E. et al.,
Genetic testing for mutations in presenilin or amyloid precursor protein, which have high
penetrance, is also available for family members suspected of having Familial
Autosomal Dominant AD, a rare but aggressive form of young-onset AD. Such testing
is also helpful in familial cases of the other neurodegenerative disorders.

Additional laboratory tests commonly used are cerebrospinal fluid (CSF)
biomarkers, which test for CSF amyloid β₁₋₄₂ (Aβ42), total tau, and phosphorylated tau.
Low CSF Aβ42, elevated total tau and increased phosphorylated tau are positive
biomarkers for AD (even prior to dementia), which reflect plaque pathology, axonal
degeneration, and intraneuronal tangles (Bouwman, F. H. et al., 2009; Mattsson, N. et al., 2012). Changes in these CSF biomarkers may assist in differential diagnosis of dementia (Hulstaert, F. et al., 1999; Schoonenboom, N. S. et al., 2012), and improve predictions for MCI patients who convert to AD (Heister, D., Brewer, J. B., Magda, S., Blennow, K., & McEvoy, L. K., 2011; Mattsson, N. et al., 2009).

1.1.7 Neuroimaging

As the focus of this thesis is based around neuroimaging of dementia, this topic will be discussed in further detail throughout this thesis. It is important to note that the only neuroimaging techniques recommended by the AAN practice parameter guidelines for dementia are limited to non-contrast CT or structural MRI, due to insufficient data regarding the validation of other imaging procedures at the time of publication (Knopman, D. S. et al., 2001). However, recent advances with other neuroimaging innovations such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and functional MRI, are becoming more widely available and commonly used in clinical research.

1.2 Alzheimer’s disease

1.2.1 Introduction

The disorder was first identified by a German psychiatrist and neuropathologist named Alois Alzheimer. In a 1906 lecture at the 37th Conference of South-West German Psychiatrists, Alzheimer described a 51 yr old female patient, called Auguste D, with a form of dementia characterized by progressive cognitive and memory impairments, hallucinations, paranoid delusions, and psychosocial incompetence. Alzheimer also reported on the histopathological findings from Auguste D’s autopsy,
where he described (and made drawings of) thick and impregnable neurofibrils which he called *neurofibrillary tangles*, the presence of dystrophic neurites clustered around a central core which he termed *senile plaques*, brain atrophy and evidence of arteriosclerotic changes.

The term *Alzheimer's disease* was coined later by Emil Kraepelin, in a 1910 German publication of the *Handbook of Psychiatry*, where he describes Alzheimer’s file on Auguste D in a chapter on senile dementia. These historical findings were later rediscovered in 1996 and published by the Department of Medical History in a 1997 issue of *The Lancet* (Maurer, K., Volk, S., & Gerbaldo, H., 1997). The Lancet’s publication included reprinted extracts of Alzheimer’s original handwritten notes, a drawing made by Alzheimer of the neurofibrillary tangles, and a rare photograph of Auguste D dated November, 1902.

Presently, with dementia estimates of over 35 million people worldwide, Alzheimer’s disease is the most common cause of dementia in the elderly. In line with Alzheimer’s early observations from over 100 yrs ago, AD can be characterized as a disruption of neural circuitry and neuron death through the formation of neurofibrillary tangles, the presence of amyloid plaques, and marked brain atrophy.

1.2.2 Neuropathology

As originally described by Alzheimer's histopathological report, AD can be characterized by the presence of what he termed *senile plaques* and *neurofibrillary tangles*. Subsequent modern day examinations have demonstrated that protein abnormalities, in the form of β-amyloid (Aβ) plaques, and the aggregation of tau proteins (neurofibrillary tangles), have become the neuropathological hallmarks of AD
(Querfurth, H. W. & LaFerla, F. M., 2010). While the highly variable distribution of amyloid in the brain shows no significant time-dependent distribution patterns, early autopsy-based studies of demented and non-demented brains suggest stage-dependent distribution patterns of neurofibrillary tangles – commonly referred to as Braak stages (Braak, H. & Braak, E., 1991).

Recent pathology results from a retrospective broad age-range (1-100yrs) autopsy study examining 2332 brains suggest that tauopathy associated with sporadic (non-familial) AD may begin 30-40 yrs before it manifests clinically, with tangles first appearing in the entorhinal cortex (and possibly in the lower brainstem) in the fourth decade (Braak, H. & Del, Tredici K., 2011; Braak, H., Thal, D. R., Ghebremedhin, E., & Del, Tredici K., 2011). These protein abnormalities detected in AD have led to a neuropathological characterization of AD as an accumulation of misfolded proteins, resulting in oxidative and inflammatory damage leading to synaptic dysfunction and a fragmentation of brain circuitry. This fragmentation of brain circuitry, particularly in the memory system (involving the hippocampus and medial temporal lobe) and the cholinergic system (originating in the basal forebrain), results in a debilitating state of cognitive dysfunction characteristic of AD (Geula, C., Mesulam, M. M., Saroff, D. M., & Wu, C. K., 1998).

1.2.3 Biomarkers & neuroimaging

Given that diagnostic confirmation of AD is currently based on post-mortem autopsy results, developing non-invasive, in-vivo neuroimaging techniques remain a significant priority for research and early diagnosis of the disease (Ashford, J. W. et al., 2011; Mueller, S., Keeser, D., Reiser, M. F., Teipel, S., & Meindl, T., 2011).
While beyond the scope of this review, there are 5 commonly used biomarkers in the study of AD (Jack, C. R., Jr. et al., 2012). Firstly, there are two measures of β-amyloid deposition: 1) CSF measures of Aβ42 (Bouwman, F. H. et al., 2009; Visser, P. J. et al., 2009), and, 2) PET amyloid imaging (Klunk, W. E. et al., 2004; Rowe, C. C. et al., 2007). Secondly, there are three measures of neuronal injury and degeneration: 3) CSF tau (Sunderland, T. et al., 2003), 4) fluorodeoxyglucose (FDG) PET (Herholz, K., 1995; Jagust, W., Reed, B., Mungas, D., Ellis, W., & Decarli, C., 2007), and, 5) structural MRI.

In addition to these commonly used biomarkers, various other functional and metabolic neuroimaging techniques have also been used to further our understanding of AD: functional MRI (BOLD fMRI) including both activation (Mandzia, J., Black, S. E., Grady, C., McAndrews, M. P., & Graham, S., 2001; Schwindt, G. C. & Black, S. E., 2008) and resting state (Greicius, M. D., Srivastava, G., Reiss, A. L., & Menon, V., 2004; Jones, D. T. et al., 2011), single photon emission tomography (SPECT) (Dougall, N. J., Bruggink, S., & Ebmeier, K. P., 2003), and nuclear magnetic resonance spectroscopy (MRS) (Kantarci, K. et al., 2007).

A recent multi-method analysis of data obtained from the Alzheimer Disease Neuroimaging Initiative (ADNI) report 93% sensitivity and 85% specificity when classifying AD from normal controls using only structural MRI (Wolz, R. et al., 2011). In this study, the classification accuracy and predictive power for early AD detection was achieved using data from a combination of various fully-automated neuroimaging techniques applied to structural MRI from the ADNI database. The following 4 neuroimaging techniques were applied and the results from these “feature extraction
methods” were integrated into a step-wise regression model: hippocampal volumetrics, cortical thickness, tensor-based morphometry, and manifold-based learning approach. Interestingly, these particular neuroimaging techniques required only a single T1-weighted MRI (ADNI T1 protocol: 1.5Tesla, 3D MPRAGE).

Although the results from this study are promising for automated differential diagnostics, they provide minimal information towards a full understanding of this elusive disease. However, the application of several neuroimaging manipulations on a minimal set of basic structural MRI, offers an encouraging methodological approach in dementia research that capitalizes on the comprehensive amounts of data which can be obtained from a single structural MRI acquisition. Additionally, the results from this ADNI-based analysis raise questions about the expense and utility of more complex and somewhat invasive biomarkers to achieve similar diagnostics (Jack, C. R., Jr., 2011; Wolz, R. et al., 2011).

While navigating through the plethora of biomarkers and imaging techniques, non-invasive structural MRI remains the time-honored standard neuroimaging tool for research in AD and aging. As demonstrated by Wolz and colleagues (2011), various analysis techniques can be derived and combined to provide a comprehensive dataset from a single T1 structural MRI acquisition (Wolz, R. et al., 2011), further enhanced by incorporating routinely obtained clinical information. Thus, a multi-method approach based on structural MRI, combined with routine clinical information, may provide sufficiently comprehensive data for research and diagnostic purposes.
1.3 Cerebrovascular disease

1.3.1 Introduction

Cerebrovascular disease (CVD) is a broad term used to describe pathological changes in the brain resulting from abnormalities in the brain’s vasculature. The study of vascular risk as related to AD may encompass a multitude of factors for consideration: hypertension, diabetes, exercise, smoking, alcohol intake, B complex vitamins, stroke, atrial fibrillation, lipids, cholesterol and diet (Purnell, C., Gao, S., Callahan, C. M., & Hendrie, H. C., 2009). These cardiovascular risk factors are believed to have some implication with cerebrovascular health and may have implications in the expression of dementia.

Of particular concern to studies on aging and dementia are the pathological processes involved with smaller arteries and arterioles, capillaries, and venules (Pantoni, L., 2010). Evidently, due to the historic work performed by Binswanger and Alzheimer (see section 1.5), previous research has focused primarily on changes in arterial vasculature (Pantoni, L. & Garcia, J. H., 1997). However, recent attention has been drawn to pathological changes associated with deep small cerebral veins (Black, S. E., Gao, F. Q., & Bilbao, J., 2009; Brown, W. R., Moody, D. M., Thore, C. R., Anstrom, J. A., & Challa, V. R., 2009; Moody, D. M., Brown, W. R., Challa, V. R., & Anderson, R. L., 1995).

1.3.2 Cerebral small vessel disease

While there are a multitude of vascular pathologies that can affect various organs and bodily functions, there are some pathological changes in the brain’s vasculature that are of particular concern in this review which will be briefly described in the
following subsections below: arteriolosclerosis, cerebral amyloid angiopathy, venous collagenosis, and inherited or genetic small vessel diseases such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). While this review is limited to sporadic forms of vascular pathologies, particularly associated with aging, some insight regarding the pathogenesis of sporadic cerebral small vessel disease may be drawn from CADASIL, a dominantly inherited disease affecting a young to middle-aged population.

In terms of clinical relevance, the risk of stroke, cognitive decline, and age-related disability, is associated with the pathological changes of cerebral small vessels (Pantoni, L., 2010). The terms “Vascular Cognitive Impairment (VCI)”, or “Vascular Dementia (VaD)”, are often used to describe cognitive impairment that is associated with vascular brain injury.

As will be discussed in Section 1.4, VaD can exist alone as a distinct pathology, or as a co-pathology with neurodegenerative conditions. When VCI is found in association with neurodegenerative processes such as AD, their combination often results in greater impairment than those having either pathology alone (Hachinski, V. et al., 2006; Schneider, J. A., 2009; Schneider, J. A., Arvanitakis, Z., Bang, W., & Bennett, D. A., 2007; Snowdon, D. A., 1997).

1.3.3 Arteriolosclerosis

Arteriolosclerosis is commonly described as a condition where there is a hardening and thickening of these vessels. Characterized by a loss of smooth muscle cells in the tunica media, deposits of fibro-hyaline material, narrowing of the lumen, and a thickening of the vessel wall, this condition is commonly associated with aging,
diabetes, and hypertension (Pantoni, L., 2010). This condition may also occur from atherosclerosis, where a hardening and thickening of larger, distal arterial blood vessels affect these smaller vessels further “downstream”.

1.3.4 Cerebral amyloid angiopathy

Cerebral amyloid angiopathy is described as a condition whereby Aβ40 deposits between the basement membrane and the walls of the blood vessels, often penetrating the smooth muscle in the walls of the vasculature (Haglund, M. & Englund, E., 2002). The accumulation of these amyloid proteins in the walls of cerebral small vessels is a pathological hallmark of AD, although this condition has also been shown to occur as in elderly individuals without dementia, familial angiopathy and Down’s syndrome (Viswanathan, A. & Greenberg, S. M., 2011).

![Figure 1.2: Microbleeds on MRI.](image)

Examples of iron deposits corresponding to old hemorrhages as evidence of cerebral amyloid angiopathy. Microbleeds shown appear as hypointense dark spots on gradient echo MRIs.
As there is some evidence to suggest that amyloid deposition is associated with degeneration of vascular components, some studies have demonstrated correlations with leukoariosis and microbleeds in the lobar regions (Fan, Y. H., Mok, V. C., Lam, W. W., Hui, A. C., & Wong, K. S., 2004; Koennecke, H. C., 2006; Pettersen, J. A. et al., 2008).

1.3.5 Venous collagenosis

Venous stenosis has only recently been given attention in the context of aging and dementia research. Recognition of this small venular disease is based primarily on the original work of Moody and colleagues (1995), who combined histology with post-mortem MRI, and found evidence to suggest a unique vasculopathy affecting the veins and venules surrounding the lateral ventricles of elderly individuals (Moody, D. M., Brown, W. R., Challa, V. R., & Anderson, R. L., 1995). Interestingly, their pathology work employed a series of histological techniques which involved the use of alkaline phosphatase staining combined with tri-chrome staining, to allow histochemical discrimination between arterioles and veins (alkaline phosphatase stains arterioles but not venules, and venular collagenosis shows up with tri-chrome staining).

In this series of studies, stenosis due to a build-up of collagen in the walls of the venular system surrounding the anterior and posterior ventricles was found in 65% of elderly individuals over the age of 65 (Brown, W. R., Moody, D. M., Challa, V. R., Thore, C. R., & Anstrom, J. A., 2002; Moody, D. M., Brown, W. R., Challa, V. R., Ghazi-Birry, H. S., & Reboussin, D. M., 1997). Moreover, the location of this venulopathy correlates with periventricular leukoariosis, which will be discussed later in this chapter.
1.3.6 Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)

CADASIL is a dominantly inherited type of small artery disease which leads to dementia and other disabilities in mid-life (Bousser, M. G. & Tournier-Lasserve, E., 1994; Tournier-Lasserve, E. et al., 1993). Originally called “hereditary multi-infarct dementia,” CADASIL is a generalized angiopathy manifesting clinically with symptoms of migraine with aura, seizures, recurrent ischemic events (stroke and transient ischemic attacks or TIAs), progressive (or stepwise) dementia, and mood disorders with severe depressive episodes (Chabriat, H. et al., 1995; Desmond, D. W. et al., 1999; Dichgans, M. et al., 1998; Herve, D. & Chabriat, H., 2010). Although the clinical presentation varies within and between affected families, a study examining 148 participants belonging to 7 French Caucasian families, report mean age onset of symptoms at 45 yrs (±10.6), with mean age of death at 64.5 yrs (±10.6) (Chabriat, H. et al., 1995). A recent review of CADASIL studies report prevalence of over 500 families worldwide (Herve, D. & Chabriat, H., 2010).

Diagnosis of CADASIL relies heavily on neuroimaging, where hyperintense signals on T2 MRI can be observed in the presymptomatic period at the age of 20. As the disease progresses to later life, these hyperintense signals appear diffuse and can involve the whole white matter, including the deep white matter as well as the so-called U-fibres directly beneath cortical gray matter, external capsule, temporal poles, corpus callosum, and brain stem (Chabriat, H. et al., 1998; Coulthard, A., Blank, S. C., Bushby, K., Kalaria, R. N., & Burn, D. J., 2000). See Figure 1.2.
Given recent commentaries which regard CADASIL as a pure form of subcortical ischemic vascular dementia, the study of this early onset vasculopathy may provide insight into the pathogenesis of sporadic small vessel disease commonly associated with aging and dementia later in life (Black, S. & Iadecola, C., 2009; Dichgans, M., 2002; Dichgans.M., 2008; Herve, D. & Chabriat, H., 2010).

**Figure 1.3: CADASIL.** Diffuse white matter hyperintensities on a two axial slices on FLAIR MRI from a CADASIL patient. Left image shows bilateral hyperintensities on anterior temporal poles extending out into the U-fibres and right image shows diffuse periventricular hyperintensities extending through bilateral external capsules.

### 1.4 Alzheimer’s disease and cerebrovascular disease

Alzheimer’s disease is the most common form of dementia, accounting for over half of cases at autopsy and in clinical series (Querfurth, H. W. & LaFerla, F. M., 2010). When AD coexists with some form of cerebrovascular injury, their combined pathologies
account for the majority of all dementias (Matthews, F. E. et al., 2009; Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS), 2001; Schneider, J. A., Arvanitakis, Z., Bang, W., & Bennett, D. A., 2007). Based on results from various cohort and population-based studies, it has been established that common vascular factors, such as hypertension, smoking, obesity, and hypercholesterolemia, constitute the majority of modifiable risk factors for dementia (Gorelick, P. B. et al., 2011).

Recent epidemiological and pathological studies show that AD is often combined with CVD (Breteler, M. M., 2000), with one large community-based study demonstrating 45% of AD patients showed vascular lesions at autopsy (Lim, A. et al., 1999). Another autopsy study conducted on Catholic sisters found prevalence of dementia in those with AD pathology was only 57%, but it was 93% for those with small vessel infarcts in the subcortical regions (Snowdon, D. A. et al., 1997). In addition, cerebrovascular risk factors such as hypertension, diabetes, and hypercholesterolemia, are common both for stroke and AD, suggesting vascular injury to be common elements in the expression of dementia (de la Torre, J. C., 2002; Launer, L. J. et al., 2000; Petrovitch, H. et al., 2000; Schmidt, R. et al., 2004; van Dijk, E. J. et al., 2004). These studies suggest that the comorbidity of AD and CVD together cause more injury than either alone to the aging human brain, leading more readily to the expression of dementia as a clinical syndrome (Breteler, M. M., 2000; Decarli, C., 2003).

Thus, given the combined debilitating contributions of AD and CVD to dementia, advances in our understanding of AD and vascular disease in late-life is necessary towards developing proper management of these vascular risk factors in mid-life and

1.5 History of subcortical hyperintensities

1.5.1 Binswanger & Alzheimer

In 1894, a Swiss professor of psychiatry, named Otto Binswanger, described a syphilitic patient with a form of dementia characterized by a progressive decline in various mental and motor functions including speech, memory, depression and personality changes, with weakness in the lower extremities and a slight hand tremor (Blass, J. P., Hoyer, S., & Nitsch, R., 1991). Post-autopsy brain pathology description revealed enlarged ventricles with thickened ependymal lining, atrophy of the sub-cortical white matter with normal appearing cerebral cortex and sparing of the U-fibres. An additional seven cases were mentioned in a footnote of the original document.

Binswanger's report, in the absence of histological analysis, was the first to suggest that this atrophy of the white matter could be associated with a reduction in perfusion due to some arteriosclerosis of the deep penetrating pia arterioles.

In 1902, a German psychiatrist and neuropathologist named Aloysius Alzheimer, provided a histological report of a similar case confirming Binswanger’s suggestion that this progressive decline in mental and motor functioning was related to ischemia. Alzheimer’s short histological report noted sub-cortical arteriosclerosis of the long penetrating arterioles and lacunar infarcts in the white matter, internal capsule, basal ganglia, thalamus and pons (Schorer, C. E., 1992). In a 1962 review of Binswanger and Alzheimer’s reports, the term “subcortical arteriosclerotic encephalopathy” was
introduced to characterize this neuropathologic entity that was thought likely to be some form of neurosyphilis (Olszewski, Jerzy, 1962).

1.5.2 Neuroimaging

Modern neuroimaging technologies, such as computed tomography (CT) and magnetic resonance imaging (MRI), allowed a revisit and re-evaluation of the neuropathologies first observed by Binswanger and Alzheimer (Pantoni, L. & Garcia, J. H., 1995; Roman, G. C., 2002). In 1972, the first clinical computed tomography (CT) machine was installed in Atkinson Morley’s Hospital in Wimbledon, England. In 1983, the first commercially used magnetic resonance imaging (MRI) machine was installed in the Department of Diagnostic Radiology at the University of Manchester Medical School.

With the introduction of these non-invasive imaging techniques, one measuring tissue density (CT) and one measuring proton density of water (MRI), pre-mortem examination of the human brain revealed subcortical signal abnormalities that were originally believed to be an \textit{in vivo} manifestation of Binswanger’s disease. These abnormalities appeared as hypodense (dark) regions on CT, and hyperintense (bright) regions on T2-weighted (T2) and proton density (PD) MRIs.

Although the presence of these signal abnormalities originally led to a diagnosis of Binswanger’s disease, this periventricular alteration was later found to appear in both clinically demented and non-demented individuals, leading to a diagnostic revision of this common neuropathology of aging and dementia as described below (Babikian, V. & Ropper, A. H., 1987; Roman, G. C., 2002). These abnormalities, commonly observed
in vivo on MRI scans obtained in the elderly, paved the way for future studies examining aging and dementia.

1.5.3 Subcortical hyperintensities: leukoariosis

In 1987, Hachinski et al., proposed the term leuko-ariosis, describing a rarefaction (ariosis) of the cerebral white (leuko) matter (Hachinski, V. C., Potter, P., & Merskey, H., 1987). Other studies offer different nomenclatures to describe the same phenomenon, emphasizing different suggested pathologies, disease characteristics, and/or appearance on different neuroimaging techniques. Some examples of the names used to describe these elusive bright spots on MRI include: unidentified bright objects (Kertesz, A. et al., 1988), leukoencephalopathy, subcortical vasculopathy, subcortical ischemic vascular disease, micro-angiopathic disease, and white matter hyperintensities. To further complicate naming conventions, several sub-classifications of these white matter pathologies have been proposed to elucidate potential pathological heterogeneities which include: periventricular and deep white hyperintensities, lacunes, and dilated Virchow-Robin perivascular spaces.

In this thesis, the term leukoariosis is used to describe this condition. These signal abnormalities will be described using the most inclusive terminology - subcortical hyperintensities (SH), describing their location beneath the cortex in the white matter or deep cerebral nuclei.

1.6 Subcortical hyperintensities

1.6.1 Aging white matter

White matter (WM), located in the subcortical regions of the brain, is generally understood to be responsible for the transmission of information from one cortical region
to another. While gray matter (GM) is mainly comprised of neural cell bodies and dendrites, WM is mainly comprised of myelinated axons and neuroglia (oligodendroglia, astrocytes, and microglia) (Wen, W., Sachdev, P. S., Li, J. J., Chen, X., & Anstey, K. J., 2008). Anatomically, there are 4 types of WM tracts: short association fibres (commonly referred to as U-fibres), long association fibres, projection fibres, and commissural fibres (Wakana, S., Jiang, H., Nagae-Poetscher, L. M., van Zijl, P. C., & Mori, S., 2004). Association fibres are considered to be cortex-cortex pathways, connecting different cortical areas, projection fibres connect cortical areas with the brainstem, spinal chord and thalamus, and commissural fibres are hemispheric connections. Projection fibres usually travel in the superior-inferior directions, for example, thalamo-cortical projections are a substrate for executive functioning. Commissural fibres, such as the corpus callosum, anterior and posterior commissures, are hemispheric connections which serve to connect the left and right hemispheres of the brain. The so-called U-fibres, located immediately below the cortical GM, are short “U-shaped” looped fibres, which allow for communication between adjacent cortical areas. In contrast, deeper within the brain, are numerous long association fibres, which serve to connect anterior-posterior and cortico-subcortical nuclei. Damage along any of these pathways, in the form of demyelination, glial cell dysfunction, cavitation and infarction, could severely compromise normal brain functioning (Wen, W., Sachdev, P. S., Li, J. J., Chen, X., & Anstey, K. J., 2008).

Various studies have examined changes in brain tissue volumes across the human lifespan (Bartzokis, G. et al., 2001; Ge, Y. et al., 2002b; Jernigan, T. L. et al., 2001; Sowell, E. R. et al., 2003). These studies suggest that changes in WM volume
are best described with a quadratic function – where WM volumes increase throughout adolescence and into adulthood, and then generally decrease after the age of 30 (Paus, T. et al., 2001).

It is also evident that observable signs of leukoariosis, appearing as hyperintense signals on T2 MRI, may begin to appear as early as 30yrs, with one large study reporting a prevalence of 51% in normal individuals in their forties (Wen, W., Sachdev, P. S., Li, J. J., Chen, X., & Anstey, K. J., 2008). The Cardiovascular Health Study, based on a large sample of community dwelling people over the age of 65, reported that only 4.4% of its 3301 participants showed no signs of white matter changes on MRI (Longstreth, W. T., Jr. et al., 1996; Longstreth, W. T., Jr. et al., 2005), suggesting this is one of the most prevalent pathologies of aging humans.

1.6.2 Clinical relevance

Despite numerous clinically-based research studies, the clinical relevance of SH remains to be fully elucidated. The most well documented risk factors for presence of SH are aging, hypertension and other cerebrovascular risk factors (Boone, K. B. et al., 1992; Liao, D. et al., 1996; Manolio, T. A. et al., 1994; Schmidt, R., 1992). SH have also been associated with cognitive decline, particularly speed of information processing and executive functions, (Decarli, C. et al., 1995; Gunning-Dixon, F. M. & Raz, N., 2000; Longstreth, W. T., Jr. et al., 1996; van Swieten, J. C. et al., 1991a), as well as physical disability, particularly gait disorders and poor motor dexterity (Masdeu, J. C. et al., 1989; Sachdev, P. S., Wen, W., Christensen, H., & Jorm, A. F., 2005; Starr, J. M. et al., 2003; Whitman, G. T., Tang, Y., Lin, A., & Baloh, R. W., 2001). In neuropsychological studies, correlations with poor attention and reduced speed of mental processing have been
consistent across many series (Boone, K. B. et al., 1992; Steingart, A. et al., 1987; Ylikoski, R. et al., 1993).

In AD patients, SH have been correlated with Mini-Mental State Examination (MMSE) scores and Clinical Dementia Ratings (CDR) scores, even after adjusting for potentially compounding variables such as age, sex, and cardiovascular risk factors (Heo, J. H. et al., 2009). Additionally, periventricular white matter changes have been reported in 48%-100% of AD cases (Brun, A. & Englund, E., 1986; Erkinjuntti, T. & Hachinski, V., 1993). However, when simultaneously considered with measures of GM and WM atrophy, SH often explain only a small proportion of the variance, usually in relation to executive function measures (Fein, G. et al., 2000; Mungas, D., Reed, B. R., Ellis, W. G., & Jagust, W. J., 2001; Swartz, R. H., Stuss, D. T., Gao, F., & Black, S. E., 2008).

1.6.3 Pathological correlates of subcortical hyperintensities

As previously discussed, cerebral small vessel disease encompasses a broad range of pathological changes in the brain. It is important to emphasize that these small vessels cannot be visualized *in vivo* with MRI scanners currently used on humans (Pantoni, L., 2010), although technological advances in 7-Tesla MRI scanners for humans suggest *in vivo* detection of microinfarcts may be possible at this high field in the near future (Jouvent, E. et al., 2011). Despite this current limitation, numerous pathological correlates suggest that the observable parenchymal lesions implied by hyperintense signals on MRI, have vascular pathophysiological origins. Some of the commonly discussed correlates of SH in the literature include: ischemic tissue damage via arteriolosclerosis and lipohyalinosis (Babikian, V. & Ropper, A. H., 1987; van
Swieten, J. C. et al., 1991b); multiple lacunar infarcts (Longstreth, W. T., Jr. et al., 1996); état criblé or dilated perivascular spaces in the absence of gliosis and infarction (Awad, I. A., Johnson, P. C., Spetzler, R. F., & Hodak, J. A., 1986); demyelination and subependymal gliosis; amyloid angiopathy (Pantoni, L. & Garcia, J. H., 1997); clasmatodendrosis from cytoplasmic swelling and vacuolation of astroglia with beading of dendrites, see Figure 1.2 (Sahlas, D. J., Bilbao, J. M., Swartz, R. H., & Black, S. E., 2002); rarefaction especially of myelin, and periventricular venous collagenosis (Black, S. E., Gao, F. Q., & Bilbao, J., 2009; Gao, F. Q. et al., 2008; Moody, D. M., Brown, W. R., Challa, V. R., & Anderson, R. L., 1995).

![Figure 1.4: Clasmatodendrosis.](image)

Historically, the parenchymal lesions evidenced by SH have been attributed to some form of arteriolar vasculopathy. Parenchymal tissue is generally supplied by arterial small vessels originating from medium-sized arteries in the sub-arachnoid region and from larger-sized arteries at the base of the brain (Pantoni, L., 2010). The arterioles penetrating the deep white matter often exist in isolation with no collaterals, such that if a penetrating arteriole occludes, the cylinder of tissue it supplies will die.
Interestingly, the white matter surrounding the ventricles has been shown to be relatively oligaemic (Brickman, A. M. et al., 2009), suggesting a particular vulnerability in this topographical region of the brain (Holland, C. M. et al., 2008). While arteriolar occlusive disease of the long penetrating vessel remains the prevailing explanation for the relatively large and confluent SH found in this region, recent work by Moody and colleagues suggest an alternative hypothesis for this periventricular leukoariosis (Moody, D. M., Brown, W. R., Challa, V. R., & Anderson, R. L., 1995). As previously described, venous collagenosis is a venous occlusive disease characterized by a build-up of collagen in the walls of veins (Moody, D. M., Brown, W. R., Challa, V. R., Ghazi-Birry, H. S., & Reboussin, D. M., 1997). This gradual stenosis is believed to result in venous leakage and perivenous edema, suggesting an alternative or cumulative explanation for the commonly observed confluent hyperintensities found in the periventricular white matter regions in aging and AD (Andersson, T., 2010; Black, S. E., Gao, F. Q., & Bilbao, J., 2009).

1.7 MRI-derived volumetrics

Despite the numerous associations reported in the literature, methodological differences - ranging from qualitative rating scales to quantitative machine learning algorithms - limit the overall consistency when attempting to study leukoariosis. Currently, there is no single methodological approach that has been applied across many clinical study populations and many imaging research centers, which may explain some of the conflicting results reported in the literature. The following section is a review of some of the more commonly used neuroimaging techniques used for SH and basic tissue quantification.
1.7.1 Basic tissue segmentation

MRIs of the brain are typically viewed as grayscale images, where intensity differences are used to discriminate different tissue types. Each digital expression of an MRI is comprised of voxels with different intensity values, analogous to pixels which make up a picture from a digital camera. Depending on the scanning protocols, the brain’s gray matter (GM), white matter (WM), cerebrospinal fluid (CSF), and pathological change, can be identified based on voxel intensity differences. Proton density (PD), T2-weighted (T2), and fluid attenuated inversion recovery (FLAIR) images are typically used to identify SH.

Whole brain voxel intensity values can be plotted on a graph commonly referred to as an intensity histogram, where several peaks indicate different brain tissue types (GM, WM, and CSF). Segmentation of an MRI is accomplished through the use of this information in order to label each voxel according to the estimated tissue type. Differences in scanner and head coil properties result in varying inhomogeneities across MRI systems; thus posing a significant challenge when developing appropriate tissue segmentation approaches. Additionally, when compared to young normal individuals, the intensity histograms of the elderly and demented often exhibit amplitude changes and decreased peak separation between tissue types (See Figure 1.5).

Specifically, there is an increased amplitude of the CSF peak in the elderly and a decreased separation between GM and WM peaks in AD which result in a significant overlap of intensities between these basic brain tissue types (Kovacevic, N. et al., 2002).
Figure 1.5: Intensity histograms of young normal, elderly normal and AD. Note increased amplitude of the CSF peak in elderly and decreased separation between GM and WM peaks in AD (Kovacevic et al., 2002).

As a further complication, the presence of brain atrophy results in an increase in sulcal CSF, which often leads to an over-erosion during T1-based brain extraction procedures (Ramírez, J. et al., 2011). Additionally, MRI scanner inhomogeneities and variations in signal-to-noise ratios present further complications when developing and testing a particular tissue segmentation approach. These issues have led to various intensity-based tissue segmentation approaches such as: probability map registration (Ashburner, J. & Friston, K. J., 2000; Wen, W. & Sachdev, P., 2004); curve-fitting (Decarli, C. et al., 1992b; Kovacevic, N. et al., 2002); artificial intelligence and clustering.
algorithms (Fein, G. et al., 2000; Gosche, K. M. et al., 1999), or some combination of these techniques.

1.7.2 Subcortical hyperintensities: appearance on MRI

Segmentation of brain tissue is further complicated in the presence of leukoaraiosis, potentially leading to erroneous volumetrics if SH are not considered. While segmentation of basic brain tissue (GM, WM, and CSF) is typically derived from contrast provided by T1-weighted images, SH identification and quantification is implemented using T2, PD, and/or FLAIR images. When T1, T2, and PD images are co-registered, SH exhibit intensities which overlap GM and CSF (see Figure 1.5); potentially leading to significant inflation of GM estimates if segmentation is performed solely on T1 voxel intensity information. Tissue misclassification has been shown to be as high as 5% in severe cases of white matter disease, where 6.4% of total GM volume could be derived from misclassified SH. Additionally, this tissue misclassification has been shown to affect 41% of the frontal regions in a group of AD patients with varying degrees of white matter disease (Levy-Cooperman, N., Ramirez, J., Lobaugh, N. J., & Black, S. E., 2008).

Additionally, current cortical thickness techniques often fail in the presence of SH and significant brain atrophy (Ramirez, J. et al., 2011). While accurate measures from MRI-derived metrics should account for SH, particularly in aging and dementia populations that are highly susceptible to white matter changes (Hachinski, V. et al., 2006), these issues pose a significant challenge in the development of a comprehensive MRI-based volumetric segmentation package.
1.8 Quantification of subcortical hyperintensities

1.8.1 Rating scales

Various visual rating scales can be used for quick estimates of SH burden (Bocti, C. et al., 2005; Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., & Zimmerman, R. A., 1987; Scheltens, P. et al., 1993; Wahlund, L. O. et al., 2001). Visual rating of SH requires a trained user to evaluate SH burden along a severity scale which can vary in complexity and clinical utility (Gao, F. Q. et al., 2011).

The Age-Related White Matter Changes (ARWMC) scale is a widely accepted reliable rating scale that can be applied to both CT and MRI (Wahlund, L. O. et al., 2001). The ARWMC scale requires users to rate the degree of white matter changes on a 4-point scale, in 10 different brain regions (5 per hemisphere): i) frontal, ii) parieto-occipital, iii) temporal, iv) infratentorial, and v) basal ganglia. It is important to note that changes in the basal ganglia were considered “white matter” lesions, even though they
were located in sub-cortical deep gray matter. Additionally, the basal ganglia region, as defined by ARWMC criteria, includes all the deep gray nuclei as well as important white matter structures such as: the thalamus, corpus striatum, internal/external capsules, and insula. Simple rating scales such as the ARWMC can be powerful tools when attempting to obtain a general sense of SH load in a large population or to quickly stratify a large group into smaller categories based on SH severity (Levy-Cooperman, N., Ramirez, J., Lobaugh, N. J., & Black, S. E., 2008).

Visual rating scales may also be used to provide an extremely rapid impression of disruption along particular white matter tracts or particular regions of interest. The Cholinergic Pathways HyperIntensities Scale (CHIPS) is an example of a rating scale which rates hyperintensities along the cortical cholinergic projection pathways (Bocti, C. et al., 2005). With the quick evaluation and rating of only 4 axial MRI slices, the CHIPS scale showed a correlation with executive measures when applied to a sample of AD patients, more so than the ARWMC scale. These results suggest that non-myelinated cholinergic fibres may be a strategic location for white matter disease, akin to the identification of strategic location of infarcts for cognition, such as the anterior dorsomedial nucleus of the thalamus (Francis, P. T., Palmer, A. M., Snape, M., & Wilcock, G. K., 1999; Swartz, R. H., Sahlas, D. J., & Black, S. E., 2003; Swartz, R. H., Stuss, D. T., Gao, F., & Black, S. E., 2008).

It has been suggested that these cholinergic WM tracts may be particularly vulnerable to leukoariosis, as the axons within this system generally lack the typical myelin sheath surrounding most WM (Selden, N. R., Gitelman, D. R., Salamon-Murayama, N., Parrish, T. B., & Mesulam, M. M., 1998). In the CHIPS scale, the lower
and higher external capsule, corona radiata, and centrum semiovale, are evaluated for SH severity using a 3-point scale. In contrast to the ARWMC, which combines several white matter tracts with the basal ganglia and thalamus, the CHIPS scale has separate ratings for a medial cholinergic pathway, which includes cingulate white matter, and a lateral pathway, which includes the external capsule and claustrum.

While visual rating scales offer a quick impression of SH, they are subjective measures which are not suitable for measuring the progression of SH (Prins, N. D. et al., 2004; van den Heuvel, D. M. et al., 2006a). MRI-derived intensity based segmentation techniques can provide a more accurate estimate of SH volume with simultaneous measures for other brain tissue volumetrics. Overall, SH segmentation techniques fall into 3 main categories using the following general approaches: i) machine learning; ii) intensity cut-off points; and iii) template based.

1.8.2 Machine learning

A commonly applied machine learning approach for SH segmentation makes use of a fuzzy C-means clustering (FCM) algorithm that is implemented to FLAIR images. FCM is an adaptive artificial intelligence technique. It is an iterative process whereby each voxel is assigned “fuzzy” membership to a predefined set of tissue classes after a training period with an expert reviewer. Its goal is to develop a reasoning level that mimics expert classifications. Another machine learning approach makes use of the k-nearest neighbor (kNN) algorithm, where each voxel is classified by a majority vote of its k nearest neighbors after a training period of known classifications (Swartz, R. H. et al., 2002). These techniques include a lesion class, where SH voxels are labeled using each respective membership rules derived from the algorithm. While machine learning
approaches offer the promise of fully-automated SH segmentation, manual checking, additional false-positive minimization techniques, and expert training of the artificial intelligence algorithm is still required (Admiraal-Behloul, F. et al., 2005; Gibson, E., Gao, F., Black, S. E., & Lobaugh, N. J., 2010).

1.8.3 Intensity cut-offs points

Intensity cut-off point approaches for SH segmentation utilize voxel information derived from intensity histograms. With this technique, voxel labeling can be derived from histograms fitted to Gaussian curves, where +3 standard deviations (SD) on a Gaussian curve can be used to label SH voxels (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005). Similarly, modal intensity cut-off points can be applied on a slice by slice basis, where 1/3 of the height of the mode value can be used as the cut-off value for SH voxel labeling (Jack, C. R., Jr. et al., 2001). Intensity cut-off approaches also require manual intervention/correction due to flow-related artifacts in the posterior limb of the internal capsule, and increased signal intensity in the deep gray nuclei and limbic cortex.

1.8.4 Template based

The Statistical Parametric Mapping (SPM) and Voxel-Based Morphometry (VBM) software packages are sophisticated applications of this approach (Ashburner, J. & Friston, K. J., 1999; Friston, K. J., Ashburner, J., Frith, C. D., Heather, J. D., & Frackowiak, R. S. J., 1995). Template-based approaches are computational processes which require coregistration of each individual scan to a normal template, a procedure commonly referred to as normalization. Templates can be derived using averaged and
scaled scans from the study sample, or downloaded from an established averaged template, such as the one provided by the Montreal Neurological Institute (MNI).

Normalization is an iterative computational process where two images are matched based on intensity differences between their corresponding regions, using linear and non-linear transformations. Linear transformation (also referred as to affine spatial transformation) matches the source image to the template across the whole brain, using rotating, shifting, zooming and sheering, to make the two images in rough alignment globally. Nonlinear transformation (also referred to as warping) can further optimize the matching between the source and template images. The warping process will selectively compress some portions of the image while expanding other regions, and tends to be more sensitive to local differences than affine transforms.

However, nonlinear functions can also squash, distort or eliminate the region of a lesion by compressing that region, and cause considerable distortion of the brain, because lesions (such as stroke) usually have a very different intensity in a patient’s scan than the corresponding area in the template. This problem can be resolved if the region of lesion is masked during the nonlinear normalization process (Brett, M., Leff, A. P., Rorden, C., & Ashburner, J., 2001), as the masked region (e.g. area of a lesion) does not influence the parameter estimation of nonlinear functions.

Additionally, a separate SH segmentation can be performed after lesion masking, by applying a voxel-wise matching process to a normal white matter probability map. Using a weighting function, SH can be re-labeled on a voxel-wise basis, where values are compared to the normal white matter template voxels (Burton, E. J. et al., 2004; Wen, W. & Sachdev, P., 2004).
However, patient populations where wide variations of ventricle size pose a significant challenge to the normalization requirements of such template based-approaches (Decarli, C. et al., 1992a; Nestor, S. M. et al., 2008). As an additional caveat to these techniques, data analysis is limited to group-wise comparisons, which do not allow for individualized volumetric profiling and analysis (Ramirez, J. et al., 2011).

1.8.5 Fluid-attenuated inversion recovery (FLAIR) imaging

As a final note regarding SH segmentation techniques, the use of FLAIR imaging for identification and quantification has increased in popularity. A FLAIR image is basically a T2 image where the CSF signal is attenuated or nulled, increasing the conspicuity and distinctness of SH relative to all other brain tissue (Jack, C. R., Jr. et al., 2001). The nulling of CSF results in SH pathology appearing as the brightest signal relative to all intracranial tissue. Thus, the intensity histograms derived from FLAIR images have an easily identifiable intensity peak for SH voxels, allowing for highly reproducible, fully-automated segmentation approaches to be implemented (Admiraal-Behloul, F. et al., 2005; Gibson, E., Gao, F., Black, S. E., & Lobaugh, N. J., 2010; Jack, C. R., Jr. et al., 2001).

However, a recent study comparing FLAIR and T2 suggest a decrease in sensitivity of FLAIR in the thalamic regions of the brain. In a blinded review of 73 demented patients, it was shown that only 55% of thalamic lesions were identified on FLAIR compared to 97% detection when using T2 images of the same subjects (Bastos Leite, A. J., van Straaten, E. C., Scheltens, P., Lycklama, G., & Barkhof, F., 2004). An
example of this decreased sensitivity of FLAIR in the thalamus is shown in Figure 1.7, with coregistered T1, PD, and T2 images.

Figure 1.7: Decreased sensitivity of FLAIR in thalamic region. Coregistered T1 (top left), T2 (top right), PD (bottom left), and FLAIR (bottom right) images showing the same slice and orientation. Arrows indicate thalamic lesion on T1, T2, and PD, which is less conspicuous and difficult to identify on FLAIR.

1.9 Subcortical hyperintensity sub-classifications

1.9.1 Deep white and periventricular

Although somewhat controversial, the most commonly applied method to sub-classify SH is to separate into periventricular (pvSH) and deep white (dwSH) hyperintensities (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005;
Sachdev, P. & Wen, W., 2005). In general, pvSH are first defined by their proximity to the ventricles and the remaining SH are then sub-classified as dwSH (See Figure 1.8).

![Figure 1.8: Periventricular and deep white hyperintensities. Coregistered PD (left) and T2 (right) images showing same slice and orientation. Blue arrow indicates dwSH (top arrow), and red arrow indicates pvSH (bottom arrow); defined by their proximity to the ventricles.](image)

The methods used to sub-classify pvSH vary across studies in the literature. The lack of a standardized methodology may be the cause of inconsistent reports in the literature. Some studies define pvSH using an arbitrary distance (eg. 13mm) from the edge of the ventricles, where any hyperintensity within this 2-dimensional (2D) border around the ventricles is classified as pvSH (Mayer, P. L. & Kier, E. L., 1991; Sachdev, P., Chen, X., & Wen, W., 2008). Others methods attempt to capture the 3-dimensional (3D) nature of SH, applying proportional distances (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005) or 3D connectivity algorithmic approaches (Ramirez, J. et al., 2011).
Despite these methodological differences, some studies have shown pvSH and dwSH to be differentially associated with GM atrophy, ventricular dilatation, cognitive, behavioural and motor deficits (De Groot, J. C. et al., 2002; Sachdev, P., Wen, W., Chen, X., & Brodaty, H., 2007; van den Heuvel, D. M. et al., 2004; van den Heuvel, D. M. et al., 2006a). Further details regarding the pvSH and dwSH controversy will be discussed in later chapters of this thesis.

1.9.2 Lacunes and dilated Virchow-Robin perivascular spaces

In addition to pvSH and dwSH, there are 2 other commonly examined SH sub-classes: cystic fluid filled lacunar-like infarcts (lacunes) and dilated perivascular Virchow-Robin spaces (VR). The term “lacune” is derived from the Latin lacuna, referring to a tiny hole, pit, or cavity. Lacunes in the brain refer to a small cystic cavity of infarcted brain tissue, most likely caused by an interruption of blood flow to the small penetrating arteries or draining veins (Brown, W. R., Moody, D. M., Thore, C. R., Anstrom, J. A., & Challa, V. R., 2009; Fisher, C. M., 1991; Mohr, J. P., 1982; Roman, G. C., 2002). Also commonly referred to as silent or covert infarcts, lacunes appear hyperintense on PD/T2 scans and hypointense (dark) on T1 scans.

In contrast, dilated VR, or perivascular spaces, appear hyperintense on T2 and isointense to CSF on PD, and are often seen in the deep WM and basal ganglia regions (Awad, I. A., Johnson, P. C., Spetzler, R. F., & Hodak, J. A., 1986). The perivascular space is a CSF filled extension of the subarachnoid space surrounding the arteries, arterioles, veins, and venules entering the brain. These enlarged perivascular spaces occasionally co-exist with gliosis and small areas of infarction, and are often seen in the white matter and basal ganglia. They appear as dots or lines, and are generally 1mm or
less in diameter. *État criblé* refers to a dilatation or widening of this space around many vessels, as first observed by Durand-Fardel in 1842 (Barkhof, F., 2004; Braffman, B. H. et al., 1988; Roman, G. C., 2002). Durand-Fardel believed this widening of the perivascular space was due to vascular congestion. See Figure 1.7 for sample images.

There is much controversy regarding the pathological significance of VR and lacunes. While dilated VR are found in normal subjects, they have also been associated with hypertension, dementia, and incidental white matter lesions. Some studies suggest they are part of the aging process (Descombes, X., Kruggel, F., Wollny, G., & Gertz, H. J., 2004; Heier, L. A. et al., 1989; MacLullich, A. M. et al., 2004; Patankar, T. F. et al., 2005; Zhu, Y. C. et al., 2010a; Zhu, Y. C. et al., 2010b).

As the term lacunar infarct implies some form of ischemia-related tissue loss, lacunes are generally believed to be less benign than dilated perivascular spaces. The large Cardiovascular Health Study conducted in the United States report lacunes to be present in 31% of all participants and 28% of those without history of stroke (Longstreth, W. T., Jr., 1998). An analysis from the Rotterdam Scan Study, a large European-based, prospective, population-based cohort study, has shown that the presence of lacunar infarcts at baseline more than doubles the risk of dementia (Vermeer, S. E. et al., 2003c). Overall, recent summaries of the various reports from these large studies suggest that lacunes are associated with aging, hypertension, increased risk of stroke, and cognitive decline (Longstreth, W. T., Jr. et al., 2005; Vermeer, S. E. et al., 2003b; Vermeer, S. E., Longstreth, W. T., Jr., & Koudstaal, P. J., 2007).
Figure 1.9: Virchow-Robin space and lacunar infarct. Top row (left to right): T1, T2 and PD showing dilated Virchow-Robin space in basal ganglia region on the same axial slice. Dilated Virchow-Robin spaces (VR) appear hyperintense on T2, and isointense to GM on PD (i.e., bright on T2, gray on PD). Shown above is an example of VR in the basal ganglia (red arrows). Bottom row (left to right): T1, T2, and PD showing thalamic lacune (blue arrows). These cystic fluid-filled cavities appear as black holes (CSF intensity) on T1, and hyperintense on PD and T2.

Unfortunately, while various studies have demonstrated some associations with these so-called silent infarcts, methodological differences in definition, detection, and description of lacunes may explain the variable reports in the literature (Potter, G. M. et al., 2010; Potter, G. M., Marlborough, F. J., & Wardlaw, J. M., 2011; Wardlaw, J. M. et al., 2008). Clearly, these recent reviews of previous literature call for an updating of lacunar descriptions and definitions through consensus-based harmonization standards.
1.9.3 Microinfarcts

As a further complication to the assessment of lacunar infarction and dilated perivascular spaces, it has been suggested that lacunes observed on standard structural MRI may be visible macroscopic indications of a more widespread underlying microscopic pathology, commonly referred to as cerebral microinfarcts (Smith, E. E., Schneider, J. A., Wardlaw, J. M., & Greenberg, S. M., 2012). Additionally, it has also been suggested that some of the previously described dilated VR in numerous studies may actually be microinfarcts (Doubal, F. N., MacLullich, A. M., Ferguson, K. J., Dennis, M. S., & Wardlaw, J. M., 2010; Patankar, T. F. et al., 2005).

As the name suggests, microinfarcts can be defined as areas of ischemia-related tissue loss that are not observable by the naked eye (i.e., requiring microscopy). Currently, cerebral microinfarcts are typically assessed using post-mortem histology techniques, routinely with the use of haematoxylin-eosin staining (Arvanitakis, Z., Leurgans, S. E., Barnes, L. L., Bennett, D. A., & Schneider, J. A., 2011; Smith, E. E., Schneider, J. A., Wardlaw, J. M., & Greenberg, S. M., 2012). In this technique, fixed brain slabs are stained and examined under a microscope for minute foci with neuronal loss, gliosis, pallor, or more cystic lesions.

While post-mortem examinations remain the standard for identification of microinfarcts as mentioned above, future advances in ultra-high field MRI (7T), and potential improvements in diffusion weighted MR imaging (DWI) show some promise for in vivo assessment of these invisible lesions (Brundel, M., de, Bresser J., van Dillen, J. J., Kappelle, L. J., & Biessels, G. J., 2012; Gass, A., Ay, H., Szabo, K., & Koroshetz, W. J., 2004; Jouvent, E. et al., 2011).
Although not fully understood, microinfarcts have been associated with cognition and memory, and commonly coexist with AD pathology (Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS), 2001). A recent report from the Honolulu Asia Aging (HAAS) found an association with post-mortem microinfarct counts and the last antemortem score on a cognitive functioning test in both demented and non-demented males (Launer, L. J., Hughes, T. M., & White, L. R., 2011). In this sample, 64% of the 436 participants had microinfarcts, with a higher percentage in the demented group (71%) compared to the non-demented (61%). While simultaneously taking into account brain atrophy, neurofibrillary tangles, and neuritic plaque counts, results from the HAAS study suggest that microinfarcts independently contribute to cognitive impairment, particularly in non-demented individuals. Interestingly, in the non-demented group of males in this study (n=292), microinfarcts were more strongly associated with atrophy and lower cognitive scores. Not surprisingly, more neurofibrillary tangles were associated with atrophy and cognition in the demented group (n=144), with microinfarcts exhibiting a more modest association with cognition.

Similar associations have been demonstrated in the Religious Orders Study, a longitudinal clinical-pathological study of aging and dementia (Arvanitakis, Z., Leurgans, S. E., Barnes, L. L., Bennett, D. A., & Schneider, J. A., 2011). Of the 425 Catholic clergymen, 30% were found to have microinfarcts at autopsy, with a higher prevalence for demented (36%) compared to non-demented individuals (25%). Using a large battery of neuropsychological tests, microinfarcts were found to be associated with
lower episodic memory, semantic memory, and perceptual speed. In contrast to the results from the HAAS study, these associations were independent of AD pathology.

A recent systematic review was conducted to summarize both conflicting and common results, as well as methodological differences found in 32 neuropathological studies examining microinfarcts in the context of vasculopathy, dementia and cognitive decline (Brundel, M., de Bresser J., van Dillen, J. J., Kappelle, L. J., & Biessels, G. J., 2012). Based on the literature to date, microinfarcts are reported to be common in both demented and non-demented elderly individuals, and are particularly present in patients with vascular dementia (62%), AD (43%), and mixed dementia patients with AD and cerebrovascular pathologies (33%).

1.10 Summary of literature

To briefly summarize, dementia constitutes a significant challenge to health care and social welfare, given our aging human population. Alzheimer disease is the most common single cause of dementia in the elderly, but frequently keeps company with vascular pathology. When Alzheimer’s pathology is combined with vascular injury, their combined pathologies often result in greater cognitive and functional impairment than either pathology alone.

Non-invasive, structural neuroimaging plays a significant role towards a greater understanding of these two pathologies. In response to the significant role of neuroimaging, there is a plethora of neuroimaging techniques which can be applied to structural MRI to provide valuable information in vivo. Gray matter, white matter, and cerebrospinal fluid volumes can be estimated using signal contrast from T1-weighted MRI. Subcortical hyperintensities, as observed on T2-weighted and proton density MRI,
can be used as surrogate macroscopic markers to estimate cerebral small vessel vasculopathy. Hyperintensities can further be subdivided by to assess potential pathological heterogeneity between periventricular and deep subcortical hyperintensities. Additionally, the presence of cystic, fluid-filled, lacunar-like infarcts within these hyperintensities can be estimated using coregistered T1-weighted MRI. Finally, regional parcellations of these volumetrics can be derived from structural information provided by T1 images using probability-based template matching or region-of-interest based computational methods. Unfortunately, until recently there was no standard, reliable, and comprehensive MRI-based neuroimaging tools which simultaneously provided all of the aforementioned volumetrics in a regionalized manner. Additionally, severely atrophic and highly variable brain scans obtained from aging and demented populations often limit the use of neuroimaging techniques that require template-matching and were developed for use in younger, less atrophied brains.

Thus, a challenge addressed in this thesis was to develop and apply an MRI-based structural neuroimaging tool which can effectively and simultaneously estimate the following information from an aging and demented population: basic parenchymal tissue volumetrics (GM, WM, CSF) to assess regional and whole brain tissue atrophy, and regional measures of MR signal abnormalities with periventricular, deep white and lacunar separation to assess potential vascular-related changes.
1.11 Thesis Outline and Hypotheses

1.11.1 Purpose

As part of the Sunnybrook Dementia study, this thesis will examine CVD and AD by using computational neuroimaging MRI techniques to further our understanding of subcortical vasculopathy in AD in comparison to age-matched elderly controls. Subcortical hyperintensities (SH), as seen on structural MRIs, will be used as a surrogate marker of CVD. To further elucidate potential differences in SH pathology, regional and SH sub-type classifications will be examined along with brain-behavior correlations with executive function, speed of mental processing, and verbal memory. A comprehensive MRI-derived neuroimaging technique for obtaining brain tissue and SH sub-types is presented, systematically validated, and applied to a moderately sized sample of AD patients and normal elderly.

1.11.2 Chapter 2: Lesion Explorer design & hypotheses

Lesion Explorer (LE) is presented in the following chapter as a semi-automated MRI-derived tissue volumetrics processing pipeline that addresses many of the previously described neuroimaging issues associated with studies examining AD and the elderly. The technique is a complex algorithm of user-guided and computer-derived automations that result in an individualized comprehensive volumetric profile which includes regionalized measures for: intracranial tissue (GM, WM, CSF); and SH sub-types (pvSH, dwSH, and lacunes). Interestingly, LE utilizes all 3 categories of the methodological approaches described earlier in this chapter: machine learning; intensity cut-off points; and templates.
The SH volumetrics and neuroimaging pipeline results were tested for internal and external validity on a sample of AD patients (n=20). For internal validation, inter- and intra-rater volumetrics were compared with the hypothesis that there will be minimal volumetric difference between and within different raters trained to use this technique. Additionally, neuroimaging results (binary segmentation images) were tested for spatial congruence; with the hypothesis that voxel to voxel matching will exhibit a significant overlap in classification. For external validation, the SH volumetric results were tested for correspondence with the results obtained from 2 visual rating scales and an alternative segmentation approach; with the hypothesis that LE volumetrics will highly correlate with visual rating and another previously published SH segmentation technique.

In contrast to many of the previously published MRI segmentation techniques, the LE processing pipeline addresses many of the neuroimaging issues by using an innovative tri-feature approach, as it is based on intensity information derived from co-registered PD-T2, and T1 images. For example, to ensure accurate brain extraction, head from brain is accomplished using PD-T2 information, prior to T1-based basic tissue segmentation (GM, WM, CSF). Alternatively, to correct for voxel misclassification due to SH, the T1-based tissue segmentation is corrected with an additional PD-T2 based lesion segmentation. Additional details regarding LE’s solutions to various neuroimaging issues are described in Chapter 2.

1.11.3 Chapter 3: Scan-rescan design & hypotheses

A short-term scan–rescan reliability study is used to test the precision of the LE processing pipeline for future longitudinal application in Chapter 3. Twenty healthy
non-demented individuals (older: n=10; younger: n=10) were scanned on the same MRI machine within a short time period (mean ISI=15.4 days). The precision of LE was tested by comparing basic tissue and SH sub-type volumetrics obtained from baseline and follow-up scans; with the hypothesis that high intraclass correlation coefficient (ICC) statistics (Shrout, P. E. & Fleiss, J. L., 2008) will be obtained for the volumetric results. Additionally, 3 different coregistration methodologies were tested for optimal correspondence.

While technique-related and random error can be expected in a short-term scan-rescan reliability test, establishing an expected error-rate prior to the design of a longitudinal study can ensure confidence in a given neuroimaging segmentation approach. While high ICCs (>0.90) have been reported in the literature for basic tissue segmentations (Mungas, D. et al., 2005), moderate to low ICCs (0.59-0.90) have been reported for SH lesion segmentation approaches used in longitudinal studies on the elderly (Mungas, D. et al., 2005; van den Heuvel, D. M. et al., 2006a; Wen, W. & Sachdev, P. S., 2004). Furthermore, the short-term scan-rescan reliability tests reported in the literature are typically based on whole brain segmentations; in contrast, the current study tested for volumetric precision within 26 brain regions to ensure greater confidence in the LE processing pipeline. Additionally, some advantages of using LE’s PD-T2 based SH segmentation is discussed relative to a FLAIR-only based approach.

1.11.4 Chapter 4: Scan-rescan design & hypotheses

The LE processing pipeline is applied to a cross-sectional sample of AD and normal elderly controls (AD: n=176; NC: n=77) in Chapter 4. Several hypotheses were
tested in this study using a variety of the comprehensive volumetric profile data provided by LE, and performance scores obtained from neuropsychological tests obtained on this sample.

Firstly, a volumetric comparison of basic tissue types was tested; with the hypothesis that ADs would show greater brain tissue atrophy and larger ventricles compared to NCs (indicated by lower GM/WM, and higher CSF/vCSF volumes in AD vs. NC). Additionally, it was predicted that ADs would have significantly larger SH and lacunar volumes.

Secondly, SH location and sub-types were correlated with performance on tasks probing executive function, speed of mental processing, and verbal memory. The following hypotheses were established based on previous reports in the literature: SH in the frontal regions would correlate with performance on an executive task; pvSH would correlate with time to complete a complex mental processing task; and SH in the left temporal lobe would correlate with performance on a verbal memory task. Total scores on the FAS fluency task (FAS) was used to assess executive function, time to perform the Trails A trail-making test (Trails A) was used to assess speed of mental processing, and the California Verbal Learning Test (CVLT) was used to assess verbal learning and memory (Lesack, 1983). Results are discussed in light of ongoing controversies regarding the relevance of SH location and volume, white matter disease, aging and dementia.

1.11.5 Chapter 5: Discussion & future directions

The final chapter positions the LE processing pipeline and the cross-sectional results in the context of the current literature on aging and dementia, showing its
contribution and potential for future applications with other neuroimaging techniques. The results of the cross-sectional study described in Chapter 4 support theories which suggest that SH location, volume, and sub-type may be differentially associated with performance on various cognitive tasks. Theories regarding arteriolar and venular pathology, white matter anatomy, and methodological differences are discussed in further detail. The reliability and validation results described in Chapter 2 and 3 demonstrate confidence in the LE pipeline, opening the door for the combination of LE with other neuroimaging techniques such as diffusion tensor imaging (DTI), tract-based spatial statistics (TBSS), and cerebrovascular reactivity (CVR). Pilot data results for future applications are also included. Finally, results of this thesis are discussed in light of our current understanding of the complex interplay between leukoariosis, AD and normal aging.
Chapter 2: Lesion Explorer

Based on published work:


The original publication is available at:
http://www.journals.elsevier.com/neuroimage/
2.1 Introduction


Although the pathophysiological origins are not fully understood, the current literature suggests that SH: are common after age 60 (Longstreth, W. T., Jr. et al., 1996); share common cerebrovascular risk factors such as diabetes and hypertension (De Leeuw, F. E. et al., 2001; Liao, D. et al., 1996; Sachdev, P., Chen, X., & Wen, W., 2008); and are associated with increased risk of cognitive decline, stroke, gait disorders and neuropsychiatric disorders (De Groot, J. C. et al., 2001; Koga, H. et al., 2009; Longstreth, W. T., Jr. et al., 1996; Longstreth, W. T., Jr. et al., 2005; Srikanth, V. et al., 2009; Vermeer, S. E. et al., 2003a; Vermeer, S. E. et al., 2003b). To further assess the effects of vascular risk factors in overt and covert cerebrovascular disease and in
dementia, consensus criteria were developed that underline the importance of accounting for SH in studies on aging (Hachinski, V. et al., 2006).

Although visual scales can provide quick ratings of SH severity on MRI (Bocti, C. et al., 2005; Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., & Zimmerman, R. A., 1987; Scheltens, P. et al., 1993; Wahlund, L. O. et al., 2001), inconsistencies in methodological properties (Mantyla, R. et al., 1997) have led some researchers to apply intensity-based segmentation techniques that provide a more accurate estimation of SH burden - as well as quantify their extent and location. However, typical T1-based tissue segmentation techniques that do not explicitly segment T2 hyperintensities can inflate other tissue volumes. Depending on the segmentation technique used, grey matter volumes can be overestimated by failing to segment the hyperintensities (Levy-Cooperman, N., Ramirez, J., Lobaugh, N. J., & Black, S. E., 2008), see Figure 2.7 for example.

Quantitative segmentation approaches have been applied to capture SH on T2, PD and FLAIR images. Some of the recent approaches in the last decade include: fuzzy clustering models that include a lesion class class (Admiraal-Behloul, F. et al., 2005; Gosche, K. M. et al., 1999); Gaussian curve fitting to determine lesion intensity cutoff points (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005); modal intensity cutoffs applied to slice-by-slice intensity histograms (Jack, C. R., Jr. et al., 2001); k-Nearest Neighbour (kNN) algorithmic combination approaches (Anbeek, P., Vincken, K. L., van Osch, M. J., Bisschops, R. H., & van der, Grond J., 2004; Seghier, M. L., Ramlackhansingh, A., Crinion, J., Leff, A. P., & Price, C. J., 2008; Swartz, R. H. et al., 2002; Wen, W., Sachdev, P. S., Li, J. J., Chen, X., & Anstey, K. J., 2008); and
coregistration to normal templates comparing the voxel-wise SH probabilities from FLAIR images to a white matter probability map using a weighting function (Burton, E. J. et al., 2004; Wen, W. & Sachdev, P. S., 2004). These approaches range from fully automated to semi-automated labour intensive processes.

Fully automated techniques offer the advantage of high reliability and are preferable for processing large scale studies. They typically require FLAIR imaging, where SH are often more conspicuous relative to standard PD/T2 images. However, FLAIR imaging is less sensitive in detecting focal thalamic lesions (Bastos Leite, A. J., van Straaten, E. C., Scheltens, P., Lycklama, G., & Barkhof, F., 2004; Jack, C. R., Jr. et al., 2001) and was not included in the multi-centre Alzheimer's Disease Neuroimaging Initiative (ADNI) acquisitions (Jack, C. R., Jr. et al., 2008). Furthermore, FLAIR imaging alone cannot disambiguate the possible heterogeneity that is implicated in SH pathology.

In an attempt to address the hypothesis of pathological heterogeneity within SH, various sub-types have been suggested for further segmentation. One common, albeit controversial, distinction is between periventricular (pvSH) and deep white (dwSH) subcortical hyperintensities (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005; Sachdev, P. & Wen, W., 2005). Although dwSH and pvSH volumes are correlated (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005), some studies have shown pvSH and dwSH to be differentially associated with: gray matter atrophy; ventricular dilatation; and cognitive, behavioural and motor/gait performance (Sachdev, P. & Wen, W., 2005; Sachdev, P. S., Wen, W., Christensen, H.,
Lack of a standardized methodology for the definition of pvSH may be the cause of inconsistent reports in the literature. The typical method to distinguish pvSH from dwSH is to create an arbitrary two-dimensional cut-off line lateral to the ventricles on axial slices in a slice by slice manner. This arbitrary line is sometimes calculated as a proportional distance from the ventricular border to the dura mater (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005), or set using an arbitrary voxel distance from the ventricle out into the centrum semiovale. Some reviewers have suggested that there may be some neuroanatomic justification for classifying SH within a 13 mm rim around the ventricle as pvSH (Mayer, P. L. & Kier, E. L., 1991; Sachdev, P., Chen, X., & Wen, W., 2008). Various other methods arbitrarily delineate pvSH from dwSH using a linear distance calculation. However, a standardized, unbiased method that recognizes the 3-dimensional nature of SH would be preferable.

Other sub-types of SH include perivascular (Virchow-Robin) spaces and cystic fluid-filled lacunar-like infarcts. Virchow-Robin (VR) spaces are CSF-filled extensions of the subarachnoid space in the sheath surrounding blood vessels. They appear as hyperintense dots or lines on T2 images, are isointense on PD and typically 1 mm or less in diameter (Awad, I. A., Johnson, P. C., Spetzler, R. F., & Hodak, J. A., 1986). Their size, shape, and differential appearance on T2 and PD allow them to be distinguished from other SH sub-types – including lacunes.

Automatic segmentation of lacunes is less common since this requires coregistration of T2/PD/FLAIR images to a T1-based segmentation, in order to identify
CSF intensity within SH. Lacunes are associated with aging, hypertension, increased risk of stroke, and are found in 11-28% of elderly (Longstreth, W. T., Jr. et al., 1998; Vermeer, S. E. et al., 2003b; Vermeer, S. E., Longstreth, W. T., Jr., & Koudstaal, P. J., 2007). These so-called silent strokes or covert infarcts, are usually defined as 3-15 mm in diameter, are hypointense on T1, and hyperintense on both T2 and PD images. Their presence is associated with increased risk of dementia and have been correlated with decreased frontal lobe glucose metabolism with positron emission tomography (PET) imaging (Reed, B. R. et al., 2004). However, without a co-registered T1-segmentation and PD-T2 contrast for comparison, lacunes and VR-spaces are difficult to quantify through volumetric segmentation with FLAIR alone.

An additional benefit of a T1-based tissue segmentation combined with PD-T2-based SH segmentation is that it allows for relative volumetric tissue comparisons for gray matter (GM), white matter (WM), ventricular-CSF (vCSF) and sulcal-CSF (sCSF). However, whole brain global volumetrics alone provide limited information, and regionalized quantification, whether ROI-based or template-based, has become a standard expectation for any MRI-based segmentation procedure.

Hence, the need for a comprehensive, individualized MRI processing pipeline that reliably segments the brain into regionalized tissue compartments and includes the various SH sub-types has become increasing important. Lesion Explorer (LE) is the final component of an MRI-based processing pipeline that was developed with these considerations. It was built upon updated versions of two previously published components: an automated T1-based tissue segmentation protocol (Kovacevic, N. et al., 2002); and the Semi-Automated Brain Region Extraction (SABRE) parcellation
procedure (Dade, L. A. et al., 2004). The LE pipeline makes use of 3 processing components that effectively allow comprehensive analysis of individual brains through the segmentation of 8 tissue classes: GM, WM, sCSF, vCSF, lacunar and non-lacunar pvSH and dwSH – tissue volumes are then parcellated into 26 SABRE brain regions. Inter-rater and inter-method reliability data is presented with validation against an alternative kNN segmentation approach and 2 different visual rating scales.

2.2 Materials and methods

2.2.1 Subjects

Images used for this study (n=20) were selected from participants in the Sunnybrook Dementia Study - a large ongoing longitudinal observational study conducted in the LC Campbell Cognitive Neurology Research Unit and the Heart & Stroke Foundation Centre for Stroke Recovery (http://www.heartandstroke-centre.strokerecovery.ca) at Sunnybrook Health Sciences Centre, a University of Toronto academic healthcare institution. See Table 2.1 for additional details.

Exclusion criteria for this sub-study included: Parkinson’s disease or other neurological diseases other than dementia, history of significant head trauma, psychotic disorders unrelated to dementia, psychoactive substance abuse and major depression. Participants in this study had a historical profile typical of AD with insidious onset of short term memory loss. All patients received a standardized comprehensive clinical evaluation. The presence of cerebrovascular risk factors was ascertained including: arterial hypertension, diabetes, hyperlipidemia, and cardiac disorders such as coronary artery disease. All patients in this study met National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer’s Disease and Related Disorders
Association criteria for probable or possible AD, (McKhann, G. et al., 1984) and Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (American Psychiatric Association, 1994) criteria for dementia.

Table 2.1: Demographics and Whole Brain Raw Volume Lesion Data

<table>
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<tr>
<th>Ss</th>
<th>Reviewed Dx</th>
<th>Sex</th>
<th>Years of Education</th>
<th>AGE</th>
<th>Rater 1 SH Volume (mm3)</th>
<th>Rater 2 SH Volume (mm3)</th>
<th>Absolute Volume Difference</th>
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</table>

### Mean: 230.85

### 2.2.2 MRI acquisition

All brain imaging data was obtained on a 1.5 Tesla GE Signa (Milwaukee, WI) system in compliance with the consensus panel imaging recommendations on VCI (Hachinski, V. et al., 2006). Three image sets were used: a T1-weighted (axial 3D
SPGR: 5 ms TE, 35 ms TR, 1 NEX, 35° flip angle, 22 x 16.5 cm FOV, 0.859 x 0.859 mm in-plane resolution, 1.2 to 1.4 mm slice thickness depending on head size), with an interleaved PD and T2 (interleaved axial dual-echo spin echo: TE of 30 and 80 ms, 3s TR, 0.5 NEX, 20 x 20 cm FOV, 0.781 x 0.781 mm in-plane resolution, 3mm slice thickness).

2.2.3 Age-Related White Matter Changes (ARWMC)

A previously published consensus-derived rating scale developed under the auspices of the European Task Force in Age-Related White Matter Changes (ARWMC) was used to test the reliability of LE against an established rating scale (Pantoni, L. et al., 2002; Wahlund, L. O. et al., 2001) (Reported κ = 0.67; Group κ = 0.89). In brief, severity of SH was rated on PD and T2-weighted MR images in five regions in each hemisphere: frontal, parieto-occipital, temporal, basal ganglia and infratentorial. SH were accepted if they appeared on both PD and T2 images and if they were at least 5 mm in diameter. Severity was graded from 0 (none) to 3 (severe) based on the appearance of the SH. A measure of global severity was derived by summing the ratings for the 5 regions.

2.2.4 MRI Processing

An overview of the image processing steps is summarized in Figure 2.1. Note that LE is an individualized procedure where each individual’s set of scans is processed singularly and thus group analysis is not a requirement for this processing pipeline. A trained operator can process one brain in approximately 1-1.5 hrs/brain, depending on scan quality (motion artifacts, image contrast, etc.). The overall MRI processing pipeline has 3 main components: Brain-Sizer, SABRE, and Lesion Explorer.
2.2.5 Component 1 - Brain-Sizer

Brain extraction and tissue segmentation were accomplished using an updated head-from-brain (HfB) procedure from previously described methods (Kovacevic, N. et al., 2002; Levy-Cooperman, N., Ramirez, J., Lobaugh, N. J., & Black, S. E., 2008). In brief, the PD and T2 images were coregistered to the T1 using a rigid body transformation. In early efforts, the transformation was obtained using the Automated Image Registration v.5.2 (AIR5) and a ratio image cost function (Woods, R. P., Grafton, S. T., Holmes, C. J., Cherry, S. R., & Mazziotta, J. C., 1998). However, this was found to result in misalignment for some subjects, and therefore FSL’s flirt tool and a normalized mutual information cost function is now used for all coregistration (Woolrich, M. W. et al., 2009). The three coregistered images were used to extract brain and subarachnoid CSF from the supratentorial cranial compartment with some manual editing to create a binary mask which was applied to the 3 coregistered images (Figure 2.1b-c). Automation of this process was accomplished using a template-guided procedure, using an in-house template that was generated by averaging 50 previously extracted brain scans using our previous method. The T1-template was coregistered to each subject’s T1 image and the inverse transformation matrix was used to move the binary template HfB mask into subject space using nearest neighbor interpolation. The spatially transformed template HfB binary mask in subject space was then smoothed using a 3D recursive Gaussian image filter (sigma=2). The PD-T2 images were intensity normalized to have values between 0 and 1. Voxels greater than 0.9995 on the transformed, smoothed template HfB binary mask and voxels greater than a
Figure 2.1: Lesion Explorer-SABRE Image processing steps (left to right): a) T1, PD and T2; b) PD and T2 are co-registered to T1-acquisition space and a binary mask (orange) is overlayed for brain extraction; c) brain and subarachnoid CSF is extracted in preparation for tissue segmentation; d) T1-segmentation (T1seg: blue=CSF, light grey=WM, dark grey=GM), Lesion Explorer segmentation (LabVol) in red overlayed on PD-T2; e) SABRE parcellations (colors represent different SABRE regions), T1-seg corrected for SH (i.e., T1seg+LabVol), corrected segmentation volumes separated into SABRE regional compartments (i.e., T1seg+LabVol+SABRE).
predefined threshold value on the intensity normalized T2 (threshold=0.35) and PD (threshold=0.37) were accepted as brain, creating each subjects' first pass HfB binary mask. Each subjects' first pass HfB mask was smoothed using a 3D recursive Gaussian image filter (sigma=2) and voxels greater than 0.5 were accepted as brain to create the subject’s final HfB mask.

The final brain extraction mask is manually checked and corrected for common brain extraction errors, such as around the optic tracts and the more superior axial slices, using in-house image editing software and/or the itk-SNAP software package (Yushkevich, P. A. et al., 2006). The manual checking step took approx. 1-10 min of user intervention.

Brain-Sizer's extraction procedure was developed to address the tendency for other brain extraction methods to remove a significant number of sCSF voxels near the brain perimeter. Most brain extraction methods (eg., FSL’s Bet, Freesurfer's MRI Watershed) operate on only the T1 image, where there is little intensity differentiation between background and sCSF (see Figure 2.2). Therefore, we developed an extraction method that operates on the PD-T2 images, where there is good differentiation between all 3 types of brain voxels (GM/WM/CSF) and background. Thus, Brain-Sizer’s method provides a more accurate brain-extraction mask that includes all brain and subarachnoid CSF voxels.

The brain extraction mask was applied to the T1 and automatically segmented
using a previously published, in-house T1-based tissue segmentation procedure (Kovacevic, N. et al., 2002). In brief, scanner inhomogeneity corrected segmentation was accomplished by fitting four Gaussian curves to local intensity histograms to derive intensity cut-offs for classifying voxels as WM, GM, or CSF.

Designation of ventricles and cerebellum removal was manually performed on the T1-segmentation image (T1seg), using in-house image editing software and/or a modified version of the itk-SNAP software package (Yushkevich, P. A. et al., 2006). The modified itk-SNAP interface is shown in Figure 2.3. The re-labelling of CSF to vCSF was accomplished by seeding and floodfilling CSF voxels on the T1seg with the T1 image for reference. This step was performed manually in order to accurately segment periventricular subcortical hyperintensities – where lesion voxels adjacent to the ventricles can often segment as CSF in fully automated procedures.
In-house modified version of the itk-SNAP software package (Yushkevich et al., 2006) showing manual removal of cerebellum. The T1 is used as the reference (left) and then used to remove the sub-tentorial brain matter from the T1-seg (right).

The manual steps for editing the T1seg for vCSF assignment and cerebellum removal took approx. 30-45 min of user intervention, with each brain voxel classified into one of 4 categories: WM, GM, vCSF and sCSF.

2.2.6 Component 2 - Semi-Automated Brain Region Extraction (SABRE)

Brain region parcellation was accomplished using an updated version of previously described methods (Dade, L. A. et al., 2004). SABRE is a quick and reliable method that was used to extract 26 brain regions proportional to individual head sizes (ICC range: 0.97-0.99 for individual tissue classes in each region). Previous studies have applied the SABRE method in studies examining multiple sclerosis (MS) and frontotemporal dementia (FTD), showing its ability to discriminate varying pathologies (Carone, D. A. et al., 2006; Chow, T. W. et al., 2007; Chow, T. W. et al., 2008b; Chow, T. W. et al., 2008a).
In brief, a set of easily identified landmarks were traced on the masked T1 images using the 3D volume render and 2D region of interest (ROI) module in ANALYZE (Biomedical Imaging Resource, Mayo foundation, Rochester, MN, USA): the central sulcus, sylvian fissure and parieto-occipital sulcus. These tracings were combined with 7 landmarks identified in 2D on the T1 image to generate a Talairach...
proportional grid system which was used to create individualized maps of 13 lobular regions in each hemisphere – resulting in a total of 26 brain regions (Figure 2.1e). The manual steps for SABRE landmark identification took approx. 20 min of user intervention. An updated version of SABRE uses an in-house modified version of itk-SNAP for landmark identification and reduces manual intervention by 5-10 min (Yushkevich, P. A. et al., 2006). The modified itk-SNAP interface is shown in Figure 2.4. Additional study-specific SABRE regions are available upon request which include: additional temporal lobe parcellations; cholinergic pathways; cingulate; thalamus.

2.2.7 Component 3 - Lesion Explorer: Subcortical Hyperintensity Segmentation

Given that prior to this component, all brain voxels have been classified as GM/WM/vCSF/sCSF, the LE segmentation can be considered as a correction of the original T1seg – where previously classified voxels were reclassified as SH using additional information from the PD-T2 images. Further segmentation into pvSH, dwSH, lacunar and non-lacunar was accomplished using automated procedures.

SH segmentation was accomplished by applying an adaptive local thresholding model that was used for SH segmentation, similar to Kovacevic et al (2002) method for dealing with inhomogeneities. The edited T1-seg (obtained from the Brain-Sizer component) was first used to mask the coregistered PD and T2 for head-from-brain and vCSF removal. The brain images were subdivided into small 3D local regions to calculate thresholds, based on intensity histograms derived from the PD and T2 images. Mean and local maxima were used in this model to estimate intensity cutoffs (T) for SH as follows:

\[ T = \text{mean} + P \cdot (\text{max}-\text{mean}) \]
Where $P$=fractional threshold (0-1), allowing the user to calibrate the model for application to different pathologies, including cerebrovascular, and varying MRI acquisition systems. The fractional threshold was set at 0.05 for both PD and T2 on the set of reliability scans used in this study. The two SH segmentations from the PD and T2 were combined using an AND operation. The output of this step is a single labeled volume containing the segmented SH (LabVol). This automated SH segmentation method provides a simple, fast, and effective segmentation providing satisfactory initial results for further processing.

Following the initial SH segmentation, many sCSF, vCSF, and choroid plexus (CP) voxels were classified as SH. These false positive classifications were minimized using the following two-stage false positive minimization procedure.

A vCSF-CP mask was generated from the edited T1seg and subjected to the following morphological operations: A 2D dilation operation (radius = 1, cross structuring element), followed by a 2D closing operation (radius = 1, ball structuring element). Any remaining “holes” in the vCSF-CP mask were filled (where holes were defined in 2D as any region that was unreachable after flood filling from the far edge of the image). The morphological dilation and closing operations ensured that partial volume voxels near the edge of the ventricles and choroid plexus voxels were included in the vCSF-CP mask (and later removed from the SH segmentation).

A sCSF-GM mask was generated using an approach based on the fuzzy C-Means (FCM) clustering algorithm (Bezdek, J. C., Hall, L. O., Clark, M. C., Goldgof, D. B., & Clarke, L. P., 1997). In brief, the FCM algorithm is an unsupervised clustering technique that is used to partition datasets into “C” different classes. Each data point is
assigned a “fuzzy” membership grade that represents the degree to which a data point belongs to each of the classes. To generate the sCSF-GM mask, the FCM algorithm was applied to the T1 image using 4 classes: background, CSF, GM and WM. The FCM results for the CSF class were thresholded, creating a CSF mask (threshold = 0.150) and any vCSF voxels (from the mask described above) were removed, creating a sCSF mask. Any voxels not connected in 3D to the largest object in the sCSF mask were also removed, as these voxels could be cystic lesion voxels that should be included in the SH segmentation and therefore excluded from the sCSF-GM mask. The GM compartment was then estimated by taking advantage of the fact that GM voxels are typically situated within a narrow region adjacent to sCSF voxels. Specifically, the sCSF-GM mask was generated by performing a 3D dilation operation (radius = 1, ball structuring element) on the sCSF mask, followed by the application of a 2D median filter (radius = 1), and finalized with a 2D dilation operation (radius = 1, cross structuring element).

Any voxel on the SH segmentation that corresponded to a vCSF-CP or sCSF-GM voxel on the mask images was reclassified as non-SH, thereby minimizing the number of false positive classifications. Finally, hyperintensities which were 3 voxels or less in size (in 3D) were removed from the segmentation to account for small artifacts and the exclusion of most Virchow-Robin spaces. Larger VR spaces typically found in the inferior region of the basal ganglia and thalamus were manually excluded if necessary. Virchow-Robin spaces were also defined by their relative intensity differences on PD and T2 images, where they appear hyperintense on T2, isointense on PD and hypointense (dark, CSF intensity) on T1 (See Figure 2.5).
Figure 2.5: Virchow-Robin perivascular spaces. (Left to right) T1, PD, T2, T1-seg overlayed onto T1 (pink=WM, turquoise=GM, blue=CSF, red=vCSF). VR perivascular spaces are typically found in the inferior region of the basal ganglia and were defined by their relative intensity differences on T1, PD, and T2. From left to right, on T1 they appear hypointense (dark), on PD they appear isointense and are relatively unambiguous, on T2 they appear hyperintense, and generally segment as CSF (blue). There are several VR spaces that can be seen on both left and right basal ganglia. VR can thus be discriminated from lacunes, which generally appear hyperintense on both PD and T2 (see Figure 2.7).

A manual checking procedure was performed by a trained operator to remove any further false positives using an in-house editing software and/or a modified version of the itk-SNAP software package (Yushkevich, P. A. et al., 2006). The manual steps for checking the SH segmentation took approx 10-20 min of user intervention.

2.2.7.1 Periventricular and deep white segmentation

An automated 3D connectivity operation (3D face connectivity, 6 connected neighbourhood) was applied to the edited SH segmentation (LabVol) to further segment pvSH from dwSH. Using the T1seg, all SH voxel clusters that were connected in 3D to the ventricles were sub-classified as pvSH and the remaining SH voxels were classified as dwSH. In this manner, all contiguous SH adjacent to the ventricles became classified as pvSH, and all discrete SH not connected to the ventricles became classified as dwSH (see Figure 2.6).
Figure 2.6: 3D Volume renders of pvSH and dwSH. 3D volume render of pvSH (red) and dwSH (blue) displayed in sagittal (left) and slightly rotated (right) 3D space. Note the red pvSH clearly appears as a single large mass surrounding the ventricles while the blue dwSH appear as several discrete masses.

2.2.7.2 Lacunar and non-lacunar

An automated operation was used to further segment SH into lacunar and non-lacunar sub-types. Using the T1seg, all SH voxels that segmented as CSF on the T1seg were identified as cystic fluid-filled lacunar-type infarcts within SH (See Figure 2.7). The remaining voxels became classified as non-lacunar.
**Figure 2.7: Combined tri-feature segmentation.** Cystic fluid-filled lacunar-type infarcts such as the one shown with the arrow appear hypointense on T1 (top left), and hyperintense on both PD (top middle) and T2 (top right). The LE pipeline component automatically segments any CSF-intensity voxels (blue voxels, bottom left) within hyperintensities (bottom middle, bottom right). An additional segmentation is performed that sub-classifies them as lacunar in both deep white (blue within red) and periventricular (purple within yellow) lesion volumes. Note that the initial T1-seg (bottom left) misclassifies some areas of WM (pink) as GM (turquoise) due to their darker intensity on T1 (top left), stressing the importance of an additional lesion segmentation to correct for this error in severe cases (Levy-Cooperman et al., 2008).

### 2.2.8 Final Output

The final output is a comprehensive volumetric profile of an individual's brain tissue volumes with regionalized segmentation data for: GM, WM, vCSF, sCSF, lacunar
and non-lacunar pvSH, lacunar and non-lacunar dwSH in each of the 26 SABRE regions. As a final note, Brain-Sizer and Lesion Explorer components were implemented using C++ and ITK (Yoo, T. S. et al., 2002).

2.2.9 Statistical analyses

The volumetric data was organized into: i) whole brain and, ii) SABRE brain regions (26 volumes of interest), for statistical analysis.

Whole brain volume inter-rater reliability was determined using two trained raters who independently checked LE segmentations from 20 AD participants with varying degrees of SH. Inter-rater statistics were generated using the intra-class correlation coefficient of reliability (ICC) (Shrout, P. E. & Fleiss, J. L., 2008). The mean absolute volume difference was also calculated for descriptive purposes. In addition, a kappa statistic-derived reliability measure, the Similarity Index (SI), was calculated to assess the spatial agreement of LE volumes generated by each rater as follows:

\[
SI = \frac{2*(\text{Rater1} \cap \text{Rater2})}{\text{Rater1} + \text{Rater2}}
\]

Where \( \text{Rater1} \cap \text{Rater2} \) refers to the pixel-wise overlap between the two raters. The SI ranges in values from 0 to 1: where 0 indicates no spatial overlap (poor reliability) and 1 indicates perfect spatial alignment (high reliability).

Whole brain inter-method reliability was determined by comparing LE volumetrics with volumetrics generated using a previously described semi-automated segmentation based on the kNN algorithmic approach (Swartz, R. H. et al., 2002; Swartz, R. H., Stuss, D. T., Gao, F., & Black, S. E., 2008), and qualitative scores generated using the
ARWMC scale (Wahlund, L. O. et al., 2001), a consensus derived, reliable rating scale of subcortical hyperintensities. The ICC was used with the kNN method comparison and Spearman rank correlation coefficients were used with the ARWMC score comparison.

SABRE brain region inter-rater reliability was assessed for 26 brain regions using the data from the whole brain analysis. ICC was calculated for each SABRE brain region across the 20 participants. Spearman correlation coefficients were used to compare volumes from an additional SABRE mask encompassing the lateral cholinergic pathways with qualitative scores generated by a fifth rater using the Cholinergic HyperIntensities Scale (CHIPS) (Bocti, C. et al., 2005).

2.3 Results

2.3.1 Whole brain results

Whole brain mean absolute volume differences between the two raters was 230 mm³ with the following results: ICC = .99, p < .0001, and mean SI = .97, indicating excellent inter-rater reliability for whole brain volumetric and pixel-wise spatial agreement (See Table 2.1 for raw data). Compared to volumes from a previously published semi-automated segmentation using a kNN algorithm, high reliability was also demonstrated (ICC = .97, p < .0001). Whole brain segmentation of pvSH volumes also yielded high reliability results (ICC = .99, p < .0001). Since the pvSH segmentation showed high reliability and the pixel-wise spatial agreement as indicated by whole brain SI was also high, the remaining dwSH required no analysis. In addition, the automated lacunar segmentation also required no analysis as their volumes yield the exact same results from both raters. When compared to independently rated scores using the
ARWMC, a significant high Spearman correlation was revealed ($r=.86$, $p<.0001$). See Table 2.2 for summary.

### Table 2.2: Reliability Tests Summary

<table>
<thead>
<tr>
<th>Reliability Test</th>
<th>Result</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Absolute Volume Difference</td>
<td>230mm³</td>
<td></td>
</tr>
<tr>
<td>Whole Brain Volumetrics</td>
<td>ICC=.99</td>
<td>p&lt;.0001</td>
</tr>
<tr>
<td>Pixel-Wise Spatial Agreement</td>
<td>SI=.97</td>
<td></td>
</tr>
<tr>
<td>Periventricular SH Volumes</td>
<td>ICC=.99</td>
<td>p&lt;.0001</td>
</tr>
<tr>
<td>Inter-Method (kNN segmentation)</td>
<td>ICC=.97</td>
<td>p&lt;.0001</td>
</tr>
<tr>
<td>Inter-Method (ARWMC rating scale)</td>
<td>$r=.86$</td>
<td>p&lt;.0001</td>
</tr>
<tr>
<td>Regional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean SABRE regions</td>
<td>ICC=.98</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td>Inter-Method (CHIPS rating scale)</td>
<td>$r=.87$</td>
<td>p&lt;.0001</td>
</tr>
</tbody>
</table>

Summary of whole brain and regional reliability tests performed by different raters on 20 AD participants with varying degrees of SH burden.

### 2.3.2 Regional Results

Mean absolute volume difference between the two raters across all SABRE regions was 13.83 mm³ with mean ICC=.98 [range=0.91- 0.99], indicating high regional inter-rater reliability. SABRE region ICC results are summarized in Table 2.3. When compared to independently rated scores using the CHIPS scale, volumes from SABRE's additional standardized cholinergic fibres region revealed a significant high Spearman correlation ($r=.87$, $p<.0001$). (Tables 2.3 and 2.4)
**Table 2.3: Regional inter-class correlation coefficients (ICC)**

<table>
<thead>
<tr>
<th>SABRE Region</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSUPF</td>
<td>0.99</td>
</tr>
<tr>
<td>LIF</td>
<td>0.99</td>
</tr>
<tr>
<td>LOBF</td>
<td>0.99</td>
</tr>
<tr>
<td>LMOBF</td>
<td>0.92</td>
</tr>
<tr>
<td>LSP</td>
<td>0.99</td>
</tr>
<tr>
<td>LIP</td>
<td>0.99</td>
</tr>
<tr>
<td>LO</td>
<td>0.99</td>
</tr>
<tr>
<td>LAT</td>
<td>0.99</td>
</tr>
<tr>
<td>LPT</td>
<td>0.99</td>
</tr>
<tr>
<td>LABGT</td>
<td>0.98</td>
</tr>
<tr>
<td>LPBGT</td>
<td>0.93</td>
</tr>
<tr>
<td>LMSF</td>
<td>0.99</td>
</tr>
<tr>
<td>LMIF</td>
<td>0.99</td>
</tr>
<tr>
<td>RSUPF</td>
<td>0.99</td>
</tr>
<tr>
<td>RIF</td>
<td>0.99</td>
</tr>
<tr>
<td>ROBF</td>
<td>0.99</td>
</tr>
<tr>
<td>RMOBF</td>
<td>0.91</td>
</tr>
<tr>
<td>RSP</td>
<td>0.99</td>
</tr>
<tr>
<td>RIP</td>
<td>0.99</td>
</tr>
<tr>
<td>ROBF</td>
<td>0.99</td>
</tr>
<tr>
<td>RAT</td>
<td>0.99</td>
</tr>
<tr>
<td>RPT</td>
<td>0.99</td>
</tr>
<tr>
<td>RABGT</td>
<td>0.97</td>
</tr>
<tr>
<td>RPBGT</td>
<td>0.99</td>
</tr>
<tr>
<td>RMSF</td>
<td>0.99</td>
</tr>
<tr>
<td>RMIF</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td><strong>0.98</strong></td>
</tr>
</tbody>
</table>

ICC in 26 SABRE brain regions from 2 independent raters. Results are based on 20 participants with AD. All ICC’s reported met minimum, p<.01 significance.
Table 2.4: Example of the volumetric profile obtained from pipeline.

<table>
<thead>
<tr>
<th>SABRE Region</th>
<th>GM</th>
<th>WM</th>
<th>CSF</th>
<th>vCSF</th>
<th>dwSH</th>
<th>dwSH-L</th>
<th>pvSH</th>
<th>pvSH-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSUPF</td>
<td>7770.5</td>
<td>14995.0</td>
<td>6062.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2081.8</td>
<td>1.8</td>
</tr>
<tr>
<td>LIF</td>
<td>15849.3</td>
<td>24849.0</td>
<td>12816.7</td>
<td>1240.7</td>
<td>38.1</td>
<td>0.0</td>
<td>8292.5</td>
<td>422.7</td>
</tr>
<tr>
<td>LOB</td>
<td>2369.8</td>
<td>7733.2</td>
<td>4012.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>105.5</td>
<td>1.8</td>
</tr>
<tr>
<td>LMOBF</td>
<td>2404.3</td>
<td>6162.8</td>
<td>3993.4</td>
<td>2.7</td>
<td>0.0</td>
<td>0.0</td>
<td>95.7</td>
<td>2.7</td>
</tr>
<tr>
<td>LSP</td>
<td>20008.4</td>
<td>31628.7</td>
<td>10235.1</td>
<td>51.4</td>
<td>54.1</td>
<td>0.0</td>
<td>6041.4</td>
<td>8.9</td>
</tr>
<tr>
<td>LIP</td>
<td>25435.7</td>
<td>30310.9</td>
<td>10348.5</td>
<td>14646.7</td>
<td>179.0</td>
<td>0.0</td>
<td>12868.1</td>
<td>180.8</td>
</tr>
<tr>
<td>LSUPF</td>
<td>14674.2</td>
<td>31570.2</td>
<td>8312.8</td>
<td>1044.9</td>
<td>2.7</td>
<td>0.9</td>
<td>6512.0</td>
<td>16.8</td>
</tr>
<tr>
<td>LIF</td>
<td>3930.4</td>
<td>14158.4</td>
<td>7534.7</td>
<td>42.5</td>
<td>0.0</td>
<td>0.0</td>
<td>1589.9</td>
<td>0.0</td>
</tr>
<tr>
<td>LOL</td>
<td>25941.7</td>
<td>49416.2</td>
<td>21673.7</td>
<td>12047.4</td>
<td>244.6</td>
<td>0.0</td>
<td>1903.6</td>
<td>0.0</td>
</tr>
<tr>
<td>LOB</td>
<td>2756.2</td>
<td>4584.5</td>
<td>3842.7</td>
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<td>0.0</td>
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<td>0.0</td>
</tr>
<tr>
<td>LMOBF</td>
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<td>4962.0</td>
<td>101.9</td>
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<td>0.0</td>
<td>0.0</td>
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<td>29.2</td>
</tr>
<tr>
<td>LMIF</td>
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<td>12900.0</td>
<td>8955.4</td>
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<td>0.0</td>
<td>0.0</td>
<td>1415.3</td>
<td>29.2</td>
</tr>
<tr>
<td>RIF</td>
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<td>13006.3</td>
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<td>0.0</td>
<td>10683.5</td>
<td>340.3</td>
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<tr>
<td>ROB</td>
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<td>6936.5</td>
<td>4972.6</td>
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<td>0.0</td>
<td>0.0</td>
<td>14.2</td>
<td>0.0</td>
</tr>
<tr>
<td>RMOBF</td>
<td>3410.2</td>
<td>7639.3</td>
<td>3636.2</td>
<td>0.9</td>
<td>14.2</td>
<td>0.0</td>
<td>214.5</td>
<td>15.1</td>
</tr>
<tr>
<td>RSP</td>
<td>17167.2</td>
<td>30720.3</td>
<td>11373.9</td>
<td>16.0</td>
<td>8.9</td>
<td>0.0</td>
<td>7377.0</td>
<td>42.5</td>
</tr>
<tr>
<td>RIF</td>
<td>21746.3</td>
<td>27044.2</td>
<td>8349.2</td>
<td>11723.9</td>
<td>133.8</td>
<td>0.0</td>
<td>11907.4</td>
<td>70.9</td>
</tr>
<tr>
<td>RO</td>
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<td>31329.1</td>
<td>8023.9</td>
<td>901.3</td>
<td>0.0</td>
<td>0.0</td>
<td>6730.9</td>
<td>24.8</td>
</tr>
<tr>
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<td>13304.1</td>
<td>7501.1</td>
<td>22.2</td>
<td>4.4</td>
<td>4.4</td>
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<td>0.0</td>
</tr>
<tr>
<td>RPT</td>
<td>26343.2</td>
<td>50451.3</td>
<td>23291.9</td>
<td>13313.8</td>
<td>16.8</td>
<td>0.0</td>
<td>2498.3</td>
<td>4.4</td>
</tr>
<tr>
<td>RABGT</td>
<td>3906.5</td>
<td>6953.4</td>
<td>313.7</td>
<td>3973.9</td>
<td>0.0</td>
<td>0.0</td>
<td>307.5</td>
<td>1.8</td>
</tr>
<tr>
<td>RPBGT</td>
<td>3278.2</td>
<td>3474.9</td>
<td>104.6</td>
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<td>0.0</td>
<td>14.2</td>
<td>0.0</td>
</tr>
<tr>
<td>RMSF</td>
<td>6074.2</td>
<td>12248.6</td>
<td>7659.7</td>
<td>0.0</td>
<td>7.1</td>
<td>0.0</td>
<td>1591.7</td>
<td>0.9</td>
</tr>
<tr>
<td>RMIF</td>
<td>7605.6</td>
<td>12819.3</td>
<td>5303.2</td>
<td>5712.6</td>
<td>0.0</td>
<td>0.0</td>
<td>2454.9</td>
<td>242.8</td>
</tr>
</tbody>
</table>

Discrete Lesion No. | 1 | 2 | 3 | 4 | 5
---|---|---|---|---|---
Volume (mm³) | 768.36 | 665.56 | 4.43 | 4.43 | 2.66
Location (Final Slice) | 95 | 91 | 39 | 48 | 53

An example of an individual’s volumetrics profile generated using the LE pipeline. Top table shows raw segmentation volumetrics in mm³ for SABRE parcellated brain regions. SH is separated into periventricular (pvSH) and deep white (dwSH) with lacunar segmentations (pvSH-L and dwSH-L) for each SH sub-category. The supplementary table below shows discrete lacunar counts for the same subject providing size and location information for each.
2.4 Discussion

Lesion Explorer is the final component of a comprehensive segmentation and parcellation package that provides an individualized volumetric profile from standard structural MRI. The overall MRI volumetrics package is a reliable application that may be used with confidence in aging populations for both cross-sectional, and longitudinal studies with standard structural acquisition protocol.

The brain extraction component, Brain-Sizer, provides an accurate measure of an individual’s total intracranial capacity. An accurate intracranial volume is a significant and important measure as it is used for head size correction. Statistically significant differences may become not significant after correcting for head size. The large Framingham Heart Study, demonstrated this phenomenon, where men had significantly greater brain volumes as compared to women, but these differences were generally not significant after head size correction (Decarli, C. et al., 2005). In the Framingham study, total cranial volume was obtained with operator guided manual tracing along the dura mater. The goal of Brain-Sizer was to obtain a similar brain extraction output that included all intracranial tissue (including subarachnoid CSF), while minimizing exhaustive manual tracing along the dura mater. This could only be accomplished with the introduction of PD-T2, allowing for a greater contrast difference between intracranial CSF and background voxels. All intensity-based T1 skull-stripping algorithms show the same kind of segmentation errors simply because there is little contrast between CSF and background. For certain studies and for certain patient groups with specific questions, the accuracy of most T1 based skull-stripping approaches (eg. SPM, BET, Freesurfer) may be sufficient. However, in clinical dementia populations, such as
patients with typical and atypical AD, frontotemporal degeneration and mixed dementias, focal atrophy is not uncommon, and thus, the increased accuracy of our method becomes particularly important. It is also interesting to note that despite this significant finding in the Framingham study, researchers continue to opt for quick T1-based automatic brain extraction procedures that have a tendency to erroneously label sulcal CSF voxels along the perimeter of the brain as background voxels (See Figure 2.2).

The benefits of having tri-feature information from PD-T2 and T1 images were not limited to an accurate intracranial capacity measure. Although false positive minimization is accomplished with various masking procedures that are PD-T2 based, this process was actually dependent on proper segmentation of the ventricles from prior steps in the process that required a T1 image. Additionally, the SABRE parcellation procedure is accomplished with a T1 image, which allows for individualized regional classification of lesion volumes. Another benefit to Lesion Explorer’s tri-feature segmentation is the ability to further segment subcortical hyperintensities that contain cystic fluid-filled lacunar-like infarcts in both periventricular and deep white segmentations (See Figure 2.7). This specificity cannot be accomplished with PD-T2 and/or FLAIR alone. Thus, Lesion Explorer should not be understood as an isolated PD-T2 based SH segmentation. It is a comprehensive volumetric segmentation and parcellation package which utilizes information from 3 common structural MRI. Given that Lesion Explorer was built upon two previously published segmentation (Kovacevic, N. et al., 2002) and parcellation procedures (Dade, L. A. et al., 2004), we feel that the Lesion Explorer component is the final step in the right direction, with the increasing

This tri-feature segmentation can be viewed as a limitation, given the minimal MR acquisition requirements (T1, T2 and PD) to obtain such a comprehensive volumetric profile. However, multimodal acquisition parameters attest to the limited information that a single MR acquisition can provide. Without a T1, tissue segmentations for GM, WM, CSF and ventricles could not be performed accurately, and without a coregistered PD-T2, VR spaces could not be delineated from lacunes and a proper head from brain with subarachnoid CSF measures for TIC could not be performed without a significant amount of manual intervention. Until the introduction of true simultaneous multi-parametric imaging, these are the minimal MR acquisition parameters required to obtain these results accurately with this processing pipeline.

The segmentation of SH into periventricular and deep white classifications remains controversial. The approaches to delineate pvSH are highly variable, ranging from arbitrary distance measures from the ventricles to proportional distances from the ventricles to the dura mater (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005; Mayer, P. L. & Kier, E. L., 1991; Sachdev, P. S., Wen, W., Christensen, H., & Jorm, A. F., 2005; Silbert, L. C., Nelson, C., Howieson, D. B., Moore, M. M., & Kaye, J. A., 2008). Lesion Explorer employs a novel approach that is less arbitrary relative to other approaches – any SH voxel clusters that were connected in 3D to the ventricles were sub-classified as pvSH. Although there is no neuroanatomical justification to favour this approach over other approaches, upon viewing the 3D volume render of pvSH and dwSH (see Figure 2.6), we felt this approach yielded an acceptable
segmentation of pvSH which was the least arbitrary. Future research with the underlying pathology of white matter disease may result in the re-evaluation of this and other approaches (Black, S. E., Gao, F. Q., & Bilbao, J., 2009).

In contrast to the controversial pvSH and dwSH debate, the pathological significance of the lacunar sub-classification is less ambiguous. Lacunes are believed to have a more disruptive neuropathology and are associated with hypertension and increased risk of stroke and dementia (Longstreth, W. T., Jr. et al., 1998; Reed, B. R. et al., 2004; Vermeer, S. E. et al., 2003b; Vermeer, S. E., Longstreth, W. T., Jr., & Koudstaal, P. J., 2007). These cystic fluid-filled infarctions appear as CSF intensity on the T1 image and are thus derived from a coregistered T1 segmentation with a CSF compartment (See Figure 2.7). Most automatic lesion segmentations often overlook this important sub-classification as they are solely based on FLAIR imaging segmentation approaches. Furthermore, the ability to disambiguate Virchow-Robin spaces is accomplished with the intensity difference between PD and T2 images, which also cannot be accomplished accurately with FLAIR imaging alone (See Figure 2.5).

The overall manual intervention processing time ranges from 45-75min, with minimal CPU runtime. In contrast, the FreeSurfer segmentation software package reports 20 hrs of CPU runtime (2x Intel Xeon E5420) with minimal user intervention (https://surfer.nmr.mgh.harvard.edu/fswiki/ReconAllRunTimes). However, the FreeSurfer segmentation is known to fail where white matter lesions exist, which make it less than ideal for applications on an elderly population where age-related white matter changes are common. As outlined in the release notes, the final surface may not follow GM along the perimeter of the lesion.
A fix for this known issue may be included in a future release.

As the general progression of imaging analysis has tended towards more quick and automatic approaches, it is clear that the main bottlenecks of the Lesion Explorer processing pipeline can be found where user intervention is required (ranging from 1-1.5 hrs/brain). Despite this time constraining caveat, the individualized approach and comprehensive volumetric profile that is provided with our pipeline could not be accomplished without these manual interventions. Group based and template based analyses are often difficult given the large individual variability with respect to whole brain atrophy and ventricular size that is found in aging and dementia populations, especially with focal atrophy syndromes and cerebrovascular lesions which are highly variable. In this regard, the bias in our processing pipeline is evident as it was developed in conjunction with a dementia and aging clinic - where individual characterization remains relevant.

All thresholds except for the intensity normalized PD-T2 thresholds remain fixed. The normalized PD and T2 thresholds are fixed only for a given set of acquisition parameters. We believe that patient-group specific thresholds could be determined, for example in MS, compared to white matter disease from aging and vascular pathologies, and we intend to do this in future applications. Unfortunately, we cannot attest to the robustness of our pipeline when applied to multiple sites with varying parameters as we have not tested this on a multitude of inputs. However, we have had success with this method on both 1.5T and 3T scanners as well as data from several other sites with similar acquisition parameters (Chicago, Taiwan, Sherbrooke, Hong Kong, Buffalo, and
UC San Francisco). With the 3T data, our method generally requires suppression of the signal from fat, a standard option available on most MRI scanners, to make the separation of head from brain less laborious and time-consuming.

Finally, the Lesion Explorer processing pipeline can be quite useful in conjunction with other advanced imaging techniques. For example, current research is underway which utilizes the SH segmentation, WM segmentation, and SABRE parcellations, to generate diffusivity and fractional anisotropy measures for normal appearing white matter adjacent to varying degrees of white matter disease with diffusion tensor imaging (DTI). Likewise, this method can be used to evaluate cerebral blood flow by using contrast perfusion imaging or arterial spin labeling (ASL).
Chapter 3: Scan-Rescan

Based on published work:


The original publication is available at: http://www.springerlink.com/content/105708/
3.1 Introduction

Cross-sectional broad age-range examinations have provided a relatively clear picture of whole brain changes in normal aging (Bartzokis, G. et al., 2001; Ge, Y. et al., 2002b; Ge, Y. et al., 2002a; Giedd, J. N. et al., 1996; Giorgio, A. et al., 2010; Paus, T. et al., 2001; Toga, A. W., Thompson, P. M., & Sowell, E. R., 2006). Unfortunately, the majority of cross-sectional studies are limited to whole brain segmentations or large regions of interest (ROIs), often limited to the 4 major sub-divisions of the brain (frontal, temporal, parietal, and occipital).

In contrast to cross-sectional examinations, longitudinal studies have each participant serve as their own baseline control, where change is assessed directly over repeated evaluations obtained from the same participant over time. Unfortunately, the feasibility of broad age-range longitudinal studies is limited due to the significant amount of time and resources required for such an undertaking. As a result, current longitudinal studies using serial MRI have a more focused age-range where quantitative techniques can be applied to assess volumetric changes over time.

A short-term scan-rescan reliability test can be used to examine the precision of a given technique to establish an expected error rate for assessing longitudinal changes. Brain tissue volumetric estimates for gray (GM) and white matter (WM), sulcal (sCSF) and ventricular cerebrospinal fluid (vCSF) can be obtained in vivo with the use of segmentation and regional parcellation methods applied to magnetic resonance images (MRI). Additionally, studies focused on Alzheimer Disease (AD), dementia and the elderly, often include a measure for signs of subcortical vasculopathy, which appear as subcortical hyperintensities (SH) on T2-weighted (T2), proton density (PD), and fluid
attenuated inversion recovery (FLAIR) MRIs (Hachinski, V. et al., 2006; Jack, C. R., Jr. et al., 2001).

SH can be sub-classified as periventricular (pvSH) and deep white (dwSH) (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005; Sachdev, P. & Wen, W., 2005). Additionally, lacunar infarcts can be measured when T1-weighted (T1) acquisitions are obtained for assessment (Levy-Cooperman, N., Ramirez, J., Lobaugh, N. J., & Black, S. E., 2008). Subcortical hyperintensities and lacunes have been independently associated with increased risk of cognitive decline, stroke, gait disorders, vascular risk factors, and neuropsychiatric disorders (De Groot, J. C. et al., 2001; Koga, H. et al., 2009; Longstreth, W. T., Jr. et al., 1996; Srikanth, V. et al., 2009; Vermeer, S. E. et al., 2003b). Thus, in addition to standard brain tissue segmentations (GM, WM, vCSF, sCSF), it is important to account for SH to assess the effects of vascular risk factors in aging and dementia (Hachinski, V. et al., 2006).

A number of longitudinal studies employ visual rating scales to estimate SH grade on MRI scans (De Leeuw, F. E., Barkhof, F., & Scheltens, P., 2005; Longstreth, W. T., Jr. et al., 2005; Schmidt, R., Enzinger, C., Ropele, S., Schmidt, H., & Fazekas, F., 2003; Veldink, J. H., Scheltens, P., Jonker, C., & Launer, L. J., 1998; Whitman, G. T., Tang, Y., Lin, A., & Baloh, R. W., 2001). However, it has been suggested that visual rating scales are not suitable for measuring the progression of SH, as they are subjective measures that lack the accuracy and precision that an MRI-based volumetric segmentation provides (Prins, N. D. et al., 2004; van den Heuvel, D. M. et al., 2006b). To date, there is a limited number of longitudinal research studies on the elderly that have used quantitative SH volumetric segmentation techniques, and even fewer have
tracked the longitudinal progression of these lesions simultaneously with other brain tissues in a regionalized manner.

The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), is a large population study of elderly in Europe that used a semi-automated FLAIR-based segmentation which allows for periventricular and deep white differentiation of SH (van den Heuvel, D. M. et al., 2004). It was validated with a short-term scan-rescan reliability test performed on 8 participants reporting intraclass correlation coefficients (ICC) (Shrout, P. E. & Fleiss, J. L., 2008) of 0.90 for dwSH and 0.82 for pvSH (van den Heuvel, D. M. et al., 2006a).

The Sydney Stroke Study and Path Through Life studies in Australia have a sophisticated FLAIR-based semi-automatic segmentation for SH that also has the ability to separate dwSH and pvSH (Sachdev, P., Wen, W., Chen, X., & Brodaty, H., 2007). Although a full scan-rescan reliability test was not performed for this method, 20 scans were rated twice to obtain test-retest reliability results reporting 100% correspondence. An additional test was performed on 100 scans comparing the results from the Fazekas visual rating scale (Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., & Zimmerman, R. A., 1987) with the quantitative measures reporting: ICC=0.43 for whole brain SH; ICC=0.63 for deep white; and ICC=0.59 for periventricular SH (Wen, W. & Sachdev, P., 2004).

A large research group in California utilize a variety of MRI processing techniques for longitudinal studies, one of which allows for the segmentation of GM, WM, vCSF, sCSF, SH, and lacunes (Mungas, D. et al., 2005). This T1-, PD/T2-based segmentation was evaluated using a short-term scan-rescan reliability test on
percentage data from 10 participants reporting: ICC=0.95 for GM; ICC=0.92 for WM; ICC=0.99 for vCSF; ICC=0.96 for sCSF; and ICC=0.80 for SH. No reliability data was reported for lacunar segmentation. An automated volumetric segmentation for the hippocampus was also included in this study, which was validated by a comparison with a manually traced method reporting a correlation of 0.92.

The reliability and validity of Lesion Explorer (LE) for SH segmentation has been previously documented: ICC=0.99 for whole brain; ICC=0.98 for regional; and ICC=0.99 for periventricular SH. Pixel-wise spatial congruence was also high reporting a similarity index (SI) of 0.97. The volumetrics were also compared with an alternative segmentation approach (kNN segmentation) showing high inter-method reliability (ICC=0.97) and was also shown to have high correlations with 2 visual rating scales: the Age-Related White Matter Changes (ARWMC) scale, r=0.86; and the Cholinergic Pathways Hyperintensities Scale (CHIPS), r=0.87 (Ramirez, J. et al., 2011).

The purpose of the current study was to assess the viability of Lesion Explorer as a quantitative method to examine volumetric progression of: GM, WM, vCSF, sCSF, pvSH and dwSH, and lacunar infarcts in 26 different brain regions. A short-term scan-rescan reliability test was performed on 20 participants to evaluate LE as a potential segmentation and parcellation method for future longitudinal volumetric studies.

3.2 Materials and methods

3.2.1 Subjects

Participants in this study were normal non-demented community volunteers (n=20) scanned twice on the same 1.5 Tesla GE Signa (Milwaukee, WI) system. Participants in this study were divided into 2 groups: older participants (n=10,
mean=77.7 yrs, SD=11.7, range: 56 > 91 yrs), and younger participants (n=10, mean=29.4 yrs, SD=7.1, range: 22 > 43 yrs). Eight participants received their second scan on the same day, while the other 12 received their second scan within a 50 day period (mean interscan interval=15.4 days, range: 29 min > 50 days). Demographic and interscan interval (ISI) details are summarized in Table 3.1.

**Table 3.1: A summary of participant’s age, sex, and interscan interval (ISI).**

<table>
<thead>
<tr>
<th>Ss</th>
<th>Age</th>
<th>ISI (days)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>88</td>
<td>16</td>
<td>F</td>
</tr>
<tr>
<td>O2</td>
<td>66</td>
<td>50</td>
<td>M</td>
</tr>
<tr>
<td>O3</td>
<td>91</td>
<td>30</td>
<td>F</td>
</tr>
<tr>
<td>O4</td>
<td>76</td>
<td>28</td>
<td>M</td>
</tr>
<tr>
<td>O5</td>
<td>74</td>
<td>1</td>
<td>F</td>
</tr>
<tr>
<td>O6</td>
<td>70</td>
<td>1</td>
<td>F</td>
</tr>
<tr>
<td>O7</td>
<td>88</td>
<td>1</td>
<td>F</td>
</tr>
<tr>
<td>O8</td>
<td>78</td>
<td>1</td>
<td>F</td>
</tr>
<tr>
<td>O9</td>
<td>56</td>
<td>25</td>
<td>F</td>
</tr>
<tr>
<td>O10</td>
<td>90</td>
<td>42</td>
<td>F</td>
</tr>
</tbody>
</table>

Mean(SD) 77.7(11.7) 19.5(18.3) F=8, M=2

<table>
<thead>
<tr>
<th>Ss</th>
<th>Age</th>
<th>ISI (days)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>26</td>
<td>1</td>
<td>M</td>
</tr>
<tr>
<td>Y2</td>
<td>30</td>
<td>25</td>
<td>F</td>
</tr>
<tr>
<td>Y3</td>
<td>43</td>
<td>35</td>
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<td>1</td>
<td>M</td>
</tr>
<tr>
<td>Y5</td>
<td>22</td>
<td>1</td>
<td>F</td>
</tr>
<tr>
<td>Y6</td>
<td>28</td>
<td>6</td>
<td>F</td>
</tr>
<tr>
<td>Y7</td>
<td>27</td>
<td>4</td>
<td>F</td>
</tr>
<tr>
<td>Y8</td>
<td>28</td>
<td>24</td>
<td>M</td>
</tr>
<tr>
<td>Y9</td>
<td>23</td>
<td>1</td>
<td>M</td>
</tr>
<tr>
<td>Y10</td>
<td>26</td>
<td>16</td>
<td>M</td>
</tr>
</tbody>
</table>

Mean(SD) 29.4(7.1) 11.4(12.6) F=5, M=5

### 3.2.2 MRI acquisition

All brain imaging data was obtained on a 1.5 Tesla GE Signa (Milwaukee, WI).

Three image sets were used: a T1-weighted (axial 3D SPGR: 5 ms TE, 35 ms TR, 1 NEX, 35° flip angle, 22 x 16.5 cm FOV, 0.859 x 0.859 mm in-plane resolution, 1.2 to 1.4
mm slice thickness depending on head size), with an interleaved PD and T2 (interleaved axial dual-echo spin echo: TEs of 30 and 80 ms, 3s TR, 0.5 NEX, 20 x 20 cm FOV, 0.781 x 0.781 mm in-plane resolution, 3mm slice thickness).

3.2.3 MRI Processing

Full segmentation and parcellation was performed on coregistered T1, PD and T2 images using the Lesion Explorer package described previously (Ramirez, J. et al., 2011). In brief, the Brain-Sizer component was used for coregistration, head-from-brain extraction, and segmentation to obtain WM, GM, CSF and vCSF. This brain extraction method provides a reliable output that includes a significant portion of subarachnoid CSF voxels which is important for atrophy measures and head size correction, i.e., total intracranial capacity (TIC). Tissue segmentation was accomplished using a robust T1-based automatic procedure that fits four Gaussian curves to local intensity histograms (Kovacevic, N. et al., 2002). Regional parcellation was accomplished using the Semi-Automated Brain Region Extraction (SABRE) component, which parcellates the brain into 26 brain regions proportional to individual head sizes (Dade, L. A. et al., 2004). This is an individualized semi-automatic approach where 7 landmarks (eg., Sylvian fissure, central sulcus, anterior commissure, etc.) are identified on each T1 image and a Talairach proportional grid is generated for each individual to yield 13 brain regions per hemisphere. The lesion segmentation component is an intensity based approach that combines intensity-based segmentations from PD, T2, and T1 images to obtain the following lesion sub-types: pvSH, dwSH and cystic fluid-filled lacunar-type infarcts (lacunes). The final output is a comprehensive volumetric profile for each individual with regionalized segmentation data for: GM, WM, vCSF, sCSF, dwSH, pvSH, and lacunes.
Three different coregistration methodologies were applied and examined for this scan-rescan reliability test (See Figure 3.1). In Method 1, the LE processing pipeline was applied in full to both baseline and follow-up scans individually, with no coregistration outside of native acquisition space. In Method 2, the LE processing pipeline was applied to both scans, however, the baseline head from brain (HfB) and SABRE masks were coregistered in follow-up space and applied to follow-up scans. In Method 3, the follow-up scans were coregistered to baseline space for HfB and SABRE application. All 3 methods used the LE processing pipeline, however they varied on the location of coregistration space: baseline space (Method 3), follow-up space (Method 2), or in their respective native acquisition space (Method 1).

**Figure 3.1: Different Coregistration Methodologies.** A graphical representation of the 3 different coregistration methods examined in a short-term scan-rescan reliability test.
3.2.4 Statistics

Reliability was assessed using the interclass correlation coefficient of reliability (ICC) performed on the original raw volumes for each participant (Shrout, P. E. & Fleiss, J. L., 2008). Data was collapsed across the 2 groups for analysis.

3.3 Results

Regional ICCs for GM, WM, and CSF for all 3 methods are summarized in Table 3.2. All 3 methods showed excellent ICC results, indicated high reliability regardless of coregistration location. However, Method 2 yielded slightly better ICC results over the Methods 1 and 3, with Method 2 showing no ICC values below 0.90 when compared to Method 3, and greater overall 100% correspondence (ICC=1.0) when compared to Method 1. These results indicated high scan-rescan reliability for standard tissue segmentation and SABRE regional parcellation.

Table 3.2: Regional ICCs. Showing ICC results for 3 different coregistration methods.
Individual whole brain volume differences and ICCs for vCSF, SH-TOTAL, dwSH, pvSH, and lacunes are summarized in Table 3.3. Overall, results indicate high scan-rescan reliability for ventricles and lesion segmentations as indicated by excellent ICCs for: vCSF (ICC=0.9998, p<.01), SH-TOTAL (ICC=.9998, p<.01), dwSH (ICC=0.9998, p<.01), pvSH (ICC=0.9998) and Lacunar (ICC=0.9859, p<.01).

Table 3.3: Individual whole brain volume (mm³) differences and ICCs. Summary of whole brain volume differences for each individual from baseline to followup Mean volume differences and mean ICC results are summarized for each tissue type and SH subtype. Data shows results from 20 healthy volunteers who participated in a short-term scan-rescan reliability test (older participants: O1-O10; younger participants: Y1-Y10). ICCs were calculated using raw volumes, with each participant serving as their own baseline control.

<table>
<thead>
<tr>
<th>Ss</th>
<th>vCSF</th>
<th>SH-TOTAL</th>
<th>dwSH</th>
<th>pvSH</th>
<th>Lacunar</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>450.3</td>
<td>148.8</td>
<td>143.1</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>O2</td>
<td>-164.4</td>
<td>-25.8</td>
<td>-25.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>O3</td>
<td>-1625.4</td>
<td>64.3</td>
<td>69.1</td>
<td>-4.8</td>
<td>-4.8</td>
</tr>
<tr>
<td>O4</td>
<td>-556.3</td>
<td>-201.6</td>
<td>-201.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>O5</td>
<td>-49.6</td>
<td>-1.0</td>
<td>-2.1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>O6</td>
<td>375.4</td>
<td>-266.9</td>
<td>-261.1</td>
<td>-5.8</td>
<td>-5.8</td>
</tr>
<tr>
<td>O7</td>
<td>-361.9</td>
<td>-417.7</td>
<td>-390.8</td>
<td>-26.9</td>
<td>-26.9</td>
</tr>
<tr>
<td>O8</td>
<td>1583.0</td>
<td>612.1</td>
<td>616.2</td>
<td>-4.1</td>
<td>-4.1</td>
</tr>
<tr>
<td>O10</td>
<td>-679.7</td>
<td>1.9</td>
<td>1.9</td>
<td>0.0</td>
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<tr>
<td>O11</td>
<td>974.5</td>
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<td>-337.0</td>
<td>-57.6</td>
<td>-57.6</td>
</tr>
<tr>
<td>Y1</td>
<td>-118.1</td>
<td>48.0</td>
<td>32.6</td>
<td>15.4</td>
<td>15.4</td>
</tr>
<tr>
<td>Y2</td>
<td>397.9</td>
<td>-10.6</td>
<td>-10.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Y3</td>
<td>210.9</td>
<td>-51.4</td>
<td>-52.3</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Y4</td>
<td>329.3</td>
<td>20.2</td>
<td>20.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Y5</td>
<td>57.6</td>
<td>-43.2</td>
<td>-46.1</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Y6</td>
<td>-779.6</td>
<td>-161.3</td>
<td>-161.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Y7</td>
<td>-265.0</td>
<td>2.9</td>
<td>-1.0</td>
<td>3.8</td>
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</tr>
<tr>
<td>Y8</td>
<td>770.1</td>
<td>-63.4</td>
<td>-63.4</td>
<td>0.0</td>
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</tr>
<tr>
<td>Y9</td>
<td>76.5</td>
<td>48.6</td>
<td>48.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Y10</td>
<td>-97.2</td>
<td>-18.2</td>
<td>-18.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MEAN(SD)</td>
<td>26.4(687.2)</td>
<td>-35.5(212.6)</td>
<td>-32.0(205.1)</td>
<td>3.5(14.8)</td>
<td>-3.5(14.8)</td>
</tr>
<tr>
<td>ICC</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9859</td>
</tr>
</tbody>
</table>
3.4 Discussion

The data presented in this study indicates that the LE procedure is an appropriate technique for longitudinal serial MRI research applications. High scan-rescan reliability was demonstrated for the technique’s basic tissue segmentation and SABRE regional parcellation. Additionally, excellent scan-rescan reliability results were shown for SH sub-types: periventricular, deep white, and lacunar segmentations.

These results are comparable to similar techniques reported in the literature. In a similar procedure, ICCs above 0.90 were reported for GM, WM and CSF (Mungas, D. et al., 2005). Although this group used percentage data for calculations, similar results were obtained from LE using raw volumes for each tissue compartment. Furthermore, LE displayed greater reliability for lesion segmentation as indicated by higher ICC values for all lesion volumes as well as allowing for the separate analysis of periventricular, deep white, and lacunar lesion sub-types.

When compared to FLAIR-based SH segmentations, similar results are also obtained. The PROSPER study reported similar high scan-rescan ICCs values of 0.90 for dwSH and 0.82 for pvSH (van den Heuvel, D. M. et al., 2006a). One of the major advantages of LE compared to many FLAIR-only SH segmentations is its ability to provide additional tissue segmentations (GM, WM, sCSF, and vCSF), as well as a separate segmentation for silent infarcts (lacunes). Additionally, there are some reports that FLAIR is less sensitive for detecting focal thalamic lesions (Bastos Leite, A. J., van Straaten, E. C., Scheltens, P., Lycklama, G., & Barkhof, F., 2004). Another advantage of the LE procedure is the pipeline’s ability to parcellate the brain into 26 individualized
Talairach-based sub-regions. The SABRE parcellation component showed high reliability in all sub-regions of the brain for the 3 main tissue types within each region.

As a final point, most serial MRI studies use baseline masking applied to follow-up scans but it is often unclear which location is used for coregistration. In this scan-rescan test, the data suggests that either acquisition space (baseline or follow-up) would yield acceptable results. However, the coregistration of baseline masks into follow-up space yields slightly superior correspondence, particularly in the smaller regions such as the basal ganglia, which appear to be more susceptible to rotation and/or reslicing errors.

The viability of LE for application in multi-centre studies remains to be examined. The LE processing pipeline was developed as an in-house procedure that was optimized for 1.5T applications and is largely dependent on good PD/T2 contrast and high resolution 3DSPGR T1 images.
Chapter 4: Cross-Sectional

Based on manuscript in preparation:

4.1 Introduction

Subcortical hyperintensities (SH) are commonly observed phenomenon on T2-weighted (T2), proton density (PD), and fluid attenuated inversion recovery (FLAIR) magnetic resonance images (MRIs) of the aging brain (Jack, C. R., Jr. et al., 2001; Kertesz, A. et al., 1988; Pantoni, L. & Garcia, J. H., 1995). Often referred to as leukoariosis or white matter lesions, these diffuse abnormalities appear as hyperintense bright spots on PD/T2 MRIs and are believed to reflect some form of subcortical vasculopathy (Hachinski, V. C., Potter, P., & Merskey, H., 1987). Although the pathological origins of SH and their contribution to expression of dementia remains controversial, recent studies have shown SH to be associated with cognitive function, gait disturbances, and speed of mental processing (Au, R. et al., 2006; Burton, E. J. et al., 2004; De Groot, J. C. et al., 2001; Nadkarni, N. K., McIlroy, W. E., Mawji, E., & Black, S. E., 2009; O’Brien, J. T. et al., 2002; Smith, E. E. et al., 2011; van den Heuvel, D. M. et al., 2006a).

SH can be sub-classified as periventricular (pvSH) and deep white (dwSH) (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005; Sachdev, P. & Wen, W., 2005). Additionally, cystic lacunar infarcts can also be measured if 3D T1-weighted (T1) acquisitions are obtained (Levy-Cooperman, N., Ramirez, J., Lobaugh, N. J., & Black, S. E., 2008). Thus, in addition to standard brain tissue segmentations (GM, WM, vCSF, sCSF), a segmentation should be performed for SH to understand the possible contribution of ischemic vascular injury in aging and dementia (Hachinski, V. et al., 2006).
The purpose of the present study is to better understand the relationships between SH location/volume and cognitive performance in a group of Alzheimer's Disease (AD) patients and healthy elderly volunteers (NC). More specifically, this study used MRI-derived tissue volumetrics to determine if SH volumes in specific regions of the brain are associated with performance on tasks probing executive function, speed of mental processing, and verbal learning and memory.

4.2 Materials and methods

4.2.1 Subjects

MRI scans were obtained from normal elderly volunteers (NC: n=77) and Alzheimer’s Disease patients (AD: n=176) enrolled in the Sunnybrook Dementia Study - a large ongoing longitudinal study conducted in the LC Campbell Cognitive Neurology Research Unit and the Heart & Stroke Foundation Centre for Stroke Recovery (http://www.heartandstroke-centrestrokerecovery.ca) at Sunnybrook Health Sciences Centre in Toronto, Canada.

AD patients were slightly older (AD=73.7±9.1 vs. NC=70.1±8.4, p<.03), and less educated (AD=13.6±3.5 vs. NC=15.6±3.0, p<.001). The presence/non-presence of SH was not exclusionary criteria for this study, with both groups showing a non-normal distribution of SH volumes. Demographic details are shown in Table 4.1. Participants showing signs of Parkinson’s disease or neurological diseases other than dementia, history of significant head trauma, psychotic disorders unrelated to dementia, psychoactive substance abuse, and major depression, were excluded from this study. AD patients met National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) – Alzheimer’s Disease and Related Disorders Association (ADRDA)
criteria for probable or possible AD (McKhann, G. et al., 1984), and Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (American Psychiatric Association, 1994) criteria for dementia. All patients received a standardized comprehensive clinical evaluation. Normal control (NC) participants were community dwelling with no subjective or objective cognitive impairment and no history of significant psychiatric or neurological diseases. The study protocol was approved by the institutional Research Ethics Board and written informed consent was obtained from all participants and/or their caregivers/decision-makers.

4.2.2 Neuropsychological Tests

Neuropsychological testing was performed within 12 weeks of MRI acquisition and administered according to standard protocols. Total scores on the FAS fluency task (FAS) was used to assess executive function and time to perform the Trails A trail-making- test (Trails A) was used to assess speed of mental processing (Lesack, M. D., 1983). The California Verbal Learning Test (CVLT) was used to assess verbal learning and memory (Lesack, M. D., 1983). Note: higher scores on the FAS and CVLT indicated better performance while higher scores on the Trails A test implied poorer performance.

4.2.3 MRI acquisition protocols

All brain imaging data was obtained on a 1.5 Tesla GE Signa (Milwaukee, WI) system in compliance with the consensus panel imaging recommendations on VCI (Hachinski et al., 2006). Three image sets were used: a T1-weighted (axial 3D SPGR: 5 ms TE, 35 ms TR, 1 NEX, 35° flip angle, 22 x 16.5 cm FOV, 0.859 x 0.859 mm in-plane resolution, 1.2 to 1.4 mm slice thickness depending on head size), and an
interleaved PD and T2 (interleaved axial dual-echo spin echo: TEs of 30 and 80 ms, 3 s TR, 0.5 NEX, 20 x 20 cm FOV, 0.781 x 0.781 mm in-plane resolution, 3 mm slice thickness).

4.2.4 MRI processing

Full details of the image processing pipeline are previously published (Ramirez, J. et al., 2011). In brief, the Lesion Explorer (LE) processing pipeline, a tri-feature (T1, PD/T2) segmentation and parcellation procedure, was applied to obtain regionalized whole brain volumetrics for gray matter (GM), white matter (WM), sulcal cerebrospinal fluid (sCSF), ventricular CSF (vCSF), periventricular SH (pvSH), deep white SH (dwSH), and cystic fluid filled lacunar like infarcts (lacunes). The Brain-Sizer component of LE effectively removed head-from-brain to obtain a reliable measure of supra-tentorial total intracranial capacity (ST-TIC) - this process includes a measure of subarachnoid CSF. A robust T1-based basic tissue (GM, WM, sCSF) segmentation was performed, which fits localized voxel intensities to 4 Gaussian curves (Kovacevic, N. et al., 2002). The Semi-Automatic Brain Region Extraction (SABRE) component of LE parcellated the brain into 26 standardized regions of interest (ROIs). The 3rd component of LE segmented SH from PD/T2, parcellated each volume into the 26 SABRE ROIs, separated SH volumes into pvSH and dwSH using a 3D connectivity algorithm, and further segmented lacunar-like infarcts within each SH volume utilizing information from the initial T1 segmentation. The final output provided a comprehensive volumetric profile for each individual that included volumetrics within 26 SABRE ROIs for: GM, WM, sCSF, vCSF, pvSH, dwSH, and lacunes.
4.2.5 Statistical analyses

Five regression models were used to examine relationships between SH volumes and neuropsychological performance measures.

Model A: to examine the relationship between executive functioning and SH volumetrics, the 26 SABRE ROIs were combined to make 4 major subdivisions: Frontal, Temporal, Parietal, and Occipital and entered into a regression model with FAS scores. SH volumetrics were corrected for each region and expressed as a percentage of parenchymal volumes. A linear regression using backwards elimination of non-significant variables was performed on the corrected SH volumes as predictors of FAS scores (i.e., total words produced in 1 min). Age, years of education, and the brain parenchymal fraction (BPF) were entered into the regression model as covariates. BPF is a general measure of brain atrophy, defined as the total parenchymal volume divided by the total intracranial capacity (TIC).

Model B: a priori planned additional regression was performed on any significant correlations from Model A from the 4 major SABRE ROIs, to further localize the relationships, if any, within subdivisions of the major regions. Based on previous studies, it was hypothesized that frontal SH volumes would be associated with FAS scores.

Model C: To examine the relationship between speed of mental processing and SH volumetrics, corrected whole brain dwSH, pvSH and lacunar volumetrics were entered into a regression model with Trails A scores (i.e., total time taken to join the numbers). A linear regression with backwards elimination of non-significant variables was performed on corrected dwSH, pvSH, and lacunar volumetrics were entered as
predictors of Trails A scores. Age and education were entered into the regression model as covariates. Based on previous studies, it was hypothesized that pvSH would be associated with Trails A scores.

Model D: To examine the relationship between verbal memory and SH volumetrics, corrected left and right temporal lobe SH volumes were entered into a regression model with CVLT scores (i.e., total acquisition score, number of words learned after 5 trials). A linear regression with backwards elimination of non-significant variables was performed with age and education entered as covariates.

Model E: a priori planned additional regression was performed based on results from Model D, where an additional regression was performed to further localize the relationships, if any, within subdivisions of the SABRE temporal lobe ROIs. Based on previous literature, it was hypothesized that left posterior temporal SH would be associated with CVLT scores.

For group comparison analyses, SH volumetrics were non-normally distributed and thus non-parametric tests (Mann-Whitney U) for SH group comparisons as required. While raw volumetric data is presented for volumetric comparisons, the analysis was performed on head size corrected values.

4.3 Results

4.3.1 Volumetrics

Sample characteristics, between group comparisons and volumetric difference results are presented in Table 4.1. Mean raw volumetrics are presented in the table for transparency and illustrative purposes, however, head size corrected data were used
for analysis. Supra-tentorial (cerebellum was removed) total intracranial capacity (ST-TIC) raw volumes were not significantly different and relatively comparable to those reported in the literature (p<0.20, n.s.). Overall, AD patients had less parenchyma (p<0.001), less WM (p=0.002), less GM (p<0.001), more sCSF (p<0.001), and larger ventricles (p<0.001). With respect to SH comparisons, AD patients had greater SH volumes (p<0.001) which can be attributed to a greater amount of pvSH (p<0.001), rather than dwSH (p=0.16, n.s.). Although both groups had relatively minimal lacunar

### Table 4.1: Sample characteristics with between group comparisons and volumetric differences.

<table>
<thead>
<tr>
<th></th>
<th>AD (n=176)</th>
<th>NC (n=77)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>73.7 ± 9.1</td>
<td>70.1 ± 8.4</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Female sex (%)</strong></td>
<td>88 (50)</td>
<td>42 (54)</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Education, y</strong></td>
<td>13.6 ± 3.5</td>
<td>15.6 ± 3.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>AD (n=176)</th>
<th>NC (n=77)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ST-TIC</strong></td>
<td>1216.9 ± 134.6</td>
<td>1239.0 ± 108.4</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Parenchyma</strong></td>
<td>819.3 ± 99.9</td>
<td>975.5 ± 89.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>WM</strong></td>
<td>374.2 ± 77.1</td>
<td>407.4 ± 58.1</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>GM</strong></td>
<td>502.4 ± 60.9</td>
<td>562.6 ± 45.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>sCSF</strong></td>
<td>271.3 ± 66.9</td>
<td>227.9 ± 48.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>vCSF</strong></td>
<td>54.3 ± 28.7</td>
<td>35.6 ± 17.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>SH</strong></td>
<td>14.8 ± 19.5</td>
<td>5.5 ± 8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>pvSH</strong></td>
<td>13.4 ± 18.4</td>
<td>4.8 ± 7.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>dwSH</strong></td>
<td>1.03± 1.4</td>
<td>0.7 ± 0.8</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Lacunar</strong>*</td>
<td>0.4 ± 1.2</td>
<td>0.1 ± 0.2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Raw volumes (cc) are presented for transparency purposes. Analyses were performed on ST-TIC corrected data to account for differences in head size. **SH raw volumes (cc) are presented for transparency purposes, non-parametric tests were performed using corrected SH volumes expressed as a percentage of parenchymal volume. ***Lacunar volumes presented in mm$^3$.**
volumes, a significant difference was demonstrated, with AD patients showing more lacunar volumes relative to NC (p=0.01).

4.3.2 Subcortical hyperintensities and executive function: Models A&B

Model A: after backwards elimination of non-significant variables, the regression model revealed the following significant associations with FAS scores: Frontal SH volumes (b=-0.19, p<0.01) and education (b=0.19, p<0.01). Model B: based on the results from Model A, an additional analysis was performed to include 6 sub-divisions of Frontal SH using SABRE ROIs: lateral superior frontal (SF); medial superior frontal (MSF); lateral middle frontal (MF); medial middle frontal (MMF); lateral inferior frontal (IF); and medial inferior frontal (MIF). The regression model revealed the following significant associations with FAS scores: MMF (b=-0.21, p<0.001); Age (b=0.23, p<.01); education (b=0.18, p<0.01); and BPF (b=0.43, p<0.0001). Standardized b coefficients with p-values are summarized in Table 4.2. Note: b coefficients can be interpreted as the difference in factor score, where a 1% increase in SH volume in each brain region predicts the b coefficient value expressed as units of standard deviation (SD) of performance on the FAS score, for example, the results of the second analysis estimates that a 1% increase in MMF SH predicts a 0.21 standard deviation decrease in FAS score, if age, years of education, and BPF remain constant.

4.3.3 Subcortical hyperintensities and speed of mental processing: Model C

Model C: after backwards elimination of non-significant variables, the regression model revealed that pvSH volumes were associated with Trails A scores (b=0.19, p<0.01), along with Age (b=-0.18, p<0.01) and BPF (b=-5.20, p<0.0001). Standardized
b coefficients with p-values are summarized in Table 4.2. Model estimates a 1% increase in pvSH volume predicts 0.19 SD increase in Trails A time.

Table 4.2 – Summary of regression model results.

<table>
<thead>
<tr>
<th></th>
<th>Standardized b coefficient</th>
<th>$R^2$</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Executive (FAS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal SH</td>
<td>-0.19</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Education</td>
<td>0.19</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Model B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial Middle Frontal (MMF)</td>
<td>-0.21</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.23</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Education</td>
<td>0.18</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BPF</td>
<td>0.43</td>
<td>0.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Processing Speed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Trails A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pvSH</td>
<td>0.19</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>-0.18</td>
<td>0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BPF</td>
<td>-5.20</td>
<td>0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Verbal Memory (CVLT)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Temporal</td>
<td>-0.15</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>-0.31</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Education</td>
<td>0.27</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Posterior Temporal</td>
<td>-0.14</td>
<td>0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Education</td>
<td>0.27</td>
<td>0.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BPF</td>
<td>0.51</td>
<td>0.26</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

4.3.4 Subcortical hyperintensities and verbal learning and memory: Models D&E

Model D: After backwards elimination of non-significant variables, the regression model revealed the following significant associations with CVLT scores: left temporal
SH volume ($b=-0.15, p<0.01$); Age ($b=-0.31, p<0.0001$); and education ($b=0.27$, $p<0.0001$). Standardized $b$ coefficients with $p$-values are summarized in Table 4.2. Model estimates that a 1% increase in left temporal lobe SH volume predicts a 0.15 SD decrease in CVLT score. Model E: based on results from Model D, an additional analysis was performed to include anterior and posterior sub-divisions of left temporal lobe SH. Regression model revealed the following significant associations with CVLT scores: left posterior temporal ($b=-0.14, p<0.01$); education ($b=0.27, p<0.0001$) and BPF ($b=0.51, p<0.001$).

4.4 Discussion

The main results of this study support the hypothesis that location and volume of SH may be associated with executive function, processing speed, and memory. More specifically, an increase in SH within the middle inferior frontal was associated with a decline in executive functioning, an increase in SH in the periventricular regions was associated with longer psychomotor speed, and an increase in SH in the left posterior temporal lobe was associated with verbal list learning.

While the numerous reports from the literature show mixed results with whole brain SH volumetrics and associations with cognitive functioning (Au, R. et al., 2006; Carmichael, O. et al., 2010; De Groot, J. C. et al., 2001; Decarli, C. et al., 1995; Vermeer, S. E. et al., 2003a), there are limited reports that have demonstrated strategic brain-behaviour relationships with hyperintensities in specific brain regions. One study using a novel rating scale demonstrated an association with strategic SH along the cholinergic pathways and memory impairment in a group of demented participants (Bocti, C. et al., 2005). A novel study on a group of normal elderly using a combination
of structural and functional MRI demonstrated that an increase in SH within the
dorsolateral prefrontal cortex was associated with decreases in prefrontal cortex activity
during a working memory task (Nordahl, C. W. et al., 2006). Another study examining
older stroke patients using FLAIR imaging found differential associations with SH,
demonstrating SH volumes in the frontal lobe were associated with cognitive processing
speed and attention, while SH volumes in the temporal lobe were associated with
memory impairment (Burton, E. J. et al., 2004). Finally, a recent study using a voxel-
based analysis method found specific clusters of SH to be associated with executive
function and episodic memory (Smith, E. E. et al., 2011). Although the results of the
current study support this handful of studies reported in the literature, it is unclear why
more groups haven’t reported similar findings given the widespread availability of
imaging techniques which allow for SH segmentation.

It is likely that the limited number of reports found in the literature may be due to
methodological differences in quantification of SH, as well a lack of standardized
definitions for periventricular, deep white, and lacunar-like, cystic fluid-filled infarcts.
Although the increasing popularity of thinner slice 3D FLAIR images has allowed for
more automatic segmentation of SH, it has also introduced more variability as FLAIR
images have been shown to have lower sensitivity in different brain regions (Bastos
Various approaches have also been applied for the separation of periventricular from
deep white SH, where some groups have employed proportional distances from the
ventricles to the dura matter (Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust,
W. J., 2005), while other groups employ an arbitrary distance from the ventricles
The current study applied a 3D connectivity algorithm whose results will clearly vary from the previously mentioned approaches. Additionally, many older studies were based on semi-quantitative data obtained from visual rating scales to estimate SH load (Bocti, C. et al., 2005; Fazekas, F. et al., 2002; Scheltens, P. et al., 1998; Wahlund, L. O. et al., 2001), or with the use of volumetric estimates derived by adding slice by slice semi-quantitative ratings with spherical shape assumptions for each lesion (De Groot, J. C. et al., 2000). Furthermore, a recent meta-study examining differences in lacunar lesion definitions revealed a wide variation in the literature regarding the detection and classification of lacunar lesions and thus, recommended a consensus for imaging definitions for small vessel disease (Potter, G. M., Marlborough, F. J., & Wardlaw, J. M., 2011).

It is interesting to note that although the SABRE method parcellates the brain into relatively large ROIs compared to typical template matching procedures, the results from this study appear promising. The increase in the strength of association with executive function and SH volumes from Model A (Frontal: b=-1.18), to Model B (MIF: b=-5.83), demonstrates the potential for this methodology to be used in future studies. Future applications using this style of modeling should be considered when applying the LE processing pipeline.

The association with speed of mental processing and SH in the periventricular region has been reported in one major longitudinal study, which demonstrated that progression of pvSH was associated with increased time to complete a Stroop test in a large group of non-demented elderly population. Although the current study was cross-sectional, a similar association was found using the Trails A trail-making-test on a
smaller group of elderly that included a demented population. Interestingly, the volumetric table in the van den Heuvel and colleagues (2006a) study report a mean volume for pvSH at baseline of (4.12cc), which is comparable to the normal elderly pvSH volume in the current study (4.8cc).

It is likely that the contribution of AD patients in the current study drove the relationship of pvSH and mental processing speed with the added distribution of greater pvSH volumes (AD=13.4cc). This may suggest that the decrease in mental processing speed found in AD patients may in part be due to some subcortical vasculopathy in the periventricular region related to venous collagenosis (Moody, D. M., Brown, W. R., Challa, V. R., & Anderson, R. L., 1995). More specifically, a case study with pathology correlates suggested that pvSH may reflect a form of vasogenic edema resulting from venous insufficiency due to veno-occlusive disease of the deep medullary veins in the periventricular region, which may in turn, impair interstitial fluid circulation and exacerbate amyloid angiopathy that is commonly associated with AD (Black, S. E., Gao, F. Q., & Bilbao, J., 2009).

A possible explanation for this decrease in mental processing speed related to pvSH has been proposed which implicate the long association white matter tracts (De Groot, J. C. et al., 2000; van den Heuvel, D. M. et al., 2006a). Disruptions from SH in the periventricular region may affect communication between distant multiple cortical brain areas and thus resulting in an overall decrease in speed of processing (van den Heuvel, D. M. et al., 2006a). In contrast, dwSH may affect communication along the so-called “U-fibres,” that connect adjacent areas of the brain and are thus less likely to translate into a significant decrease in processing speed (De Groot, J. C. et al., 2000).
In this sample, the overall volume of dwSH was small relative to pvSH volumes and mostly non-lacunar, which may not be so pathological.

It is also important to note that while the term dwSH, for *deep white subcortical hyperintensities* was used in this study, it was chosen mainly out of convention, keeping in line with the terminology used in the literature. However, the dwSH segmentation applied in this study included hyperintensities in deep gray nuclei, such as thalamic SH, as well as those found within the deep white matter.

In this study, no brain-behavior relationships were demonstrated with lacunar lesion sub-types. As stated previously, there is a large variation in detection and identification of lacunes, consensus driven definitions have yet to be established, and recent controversies in this matter are yet to be resolved (Potter, G. M. et al., 2010; Potter, G. M., Marlborough, F. J., & Wardlaw, J. M., 2011). For clarification purposes, lacunar volumes in the current study were defined as any hypointense (CSF-intensity) voxels on T1, that are found within hyperintense SH defined voxels on PD/T2. A large number of participants in this sample population had no signs of lacunar infarction as defined by this segmentation method. Although AD patients in this study had a larger volume of lacunar infarcts compared to NC, it is likely that the small distribution and numerous zero values for lacunar lesion volumes in both groups (AD=0.4mm³±1.2; NC=0.1mm³±0.2) limited correlative power in the analysis.

The results of this study also suggest that poorer verbal learning/memory performance to be associated with SH volumes in the left posterior temporal lobe. This finding is not surprising as the left temporal lobe has traditionally been implicated with verbal learning and memory. A recent study using a similar ROI based method
examining SH volumes in a stroke population, found an association with right temporal SH and memory, using a numerical working memory task (Burton, E. J. et al., 2004). Unfortunately, the SABRE ROI subdivisions for the temporal lobes are relatively large and the posterior temporal lobe ROI embodies almost two-thirds of the entire lobe. Additional sub-divisions of the temporal lobe SABRE ROIs are currently in progress, namely: a superior and inferior sub-division; and a medial and lateral sub-division, which may be more sensitive to these relationships.

This is the first application of the LE pipeline to a moderately sized cross-sectional sample of AD and normal elderly. As this study was cross-sectional, it would be interesting to examine volumetric change over time, particularly with SH sub-types and other brain tissue compartments, to determine if these changes correlate with performance scores. Additionally, diffusion tensor imaging (DTI) measures of fractional anisotropy and diffusivity obtained on a subset of these participants would be interesting to compare with the volumetric measures.
Chapter 5: Limitations & Future Directions
5.1 Limitations

The main processing time bottleneck for the LE pipeline occurs at the stages where user-intervention is required. Improvements are currently underway which are focused on developing automated methods that mimic the results at these particular stages of the pipeline. Fortunately, there is a relatively large dataset with manual interventions at each stage that can be used as a gold standard comparison to the automated methods being developed. Automation of AC-PC alignment and additional SABRE region parcellations have been added to the pipeline and are currently being validated. Integration of this pipeline with DTI-derived white matter tractography maps are also in progress. With the introduction of novel template matching approaches and the increasing availability of parallel process computing, the final goal is to automate the LE pipeline and minimize manual user-intervention to quick quality control checking and minor editing.

While the application of this pipeline to a sample of AD and NC demonstrated various associations with SH sub-types, the study is limited by its cross-sectional design. Volumetric progression of SH along correlated with cognitive performance tasks can only truly be assessed with a longitudinal design. The scan-rescan reliability test described in Chapter 3 of this thesis validates this technique for future longitudinal studies examining SH sub-types and tissue atrophy as they progress over time, with each participant serving as their own baseline control. Analysis of longitudinal data on a subset of this sample is currently in progress. Additionally, the cross-sectional study described in Chapter 4 of this thesis was hypothesis driven, making use of only 3 neuropsychological tests: the FAS fluency task; the Trails A trail-making test; and the
CVLT verbal learning test. While beyond the scope of this thesis, a larger battery of neuropsychological and demographic data was collected on this sample, providing a rich dataset for future brain-behaviour research. Additionally, the LE pipeline provides a plethora of volumetric information which is currently being used to explore various research questions on this sample set.

The main strength of the LE processing pipeline is its modular nature, allowing it to be combined with additional imaging techniques. The LE pipeline is component-based; thus it is not a black box pipeline with an obligatory input and output. Each component is a set of complex processes which can be modulated given different input parameters which can be modified for future applications allowing for the combination of LE with other advanced imaging techniques such as diffusion tensor imaging (DTI), cerebrovascular reactivity (CVR), cortical thickness, positron emission tomography (PET) and arterial spin labeling (ASL).

The following sections will provide some details regarding the potential for future applications of the LE pipeline with other imaging techniques, as well as some pilot work describing some future analyses which can be performed using this comprehensive volumetrics and parcellation imaging pipeline.

5.2 Diffusion Tensor Imaging (DTI)

Diffusion weighted MRI (DWI) measures the diffusion characteristics of water molecules in biological tissue (Le, Bihan D. et al., 1986). This technique is commonly used to visualize acute cerebral ischemia and regional microstructural changes associated with neurodegenerative disorders (Kantarci, K. et al., 2001; Provenzale, J. M. & Sorensen, A. G., 1999).
DWI exploits the Brownian motion of water molecules to infer microstructural integrity of neural tissue (Le, Bihan D. et al., 1986). Water molecules naturally exhibit random thermal motion (Brownian motion). When there are no physical barriers to impede this motion, diffusion is isotropic, i.e., equal in all directions. In normal biological tissue, this isotropic free diffusion of water molecules is impeded by microstructural barriers (eg. cell walls, membranes, fibres, intracellular organelles) resulting in more anisotropic diffusion, i.e., unidirectional (Malloy, P., Correia, S., Stebbins, G., & Laidlaw, D. H., 2007; Mori, S. & Zhang, J., 2006). DWI estimates this motion using gradient pulses which are typically acquired along 3 axes (x, y, z). Two gradients per axis are required to estimate diffusion, one that is sensitive to the diffusion process and one that is not, resulting in a diffusion-weighted and a non-diffusion-weighted image. Based on a comparison of these two images, a diffusion coefficient is calculated for each voxel where voxels appearing bright indicate slower diffusion. Conditions with slower diffusion may indicate edema resulting from acute cerebral ischemia (Lansberg, M. G. et al., 2000; Provenzale, J. M. & Sorensen, A. G., 1999) or other pathological processes such as neurodegeneration caused by AD (Kantarci, K. et al., 2001; Kantarci, K. et al., 2005).

Unfortunately, diffusion estimates provided by DWI are rotationally variant, meaning they are dependent on the orientation of neural tissue relative to the 3 axes along which the gradients are applied. Moreover, the diffusion estimates are based on relative intensity values of each voxel. This led to the development of diffusion tensor imaging (DTI), which is based on 6 or more diffusion weighted images acquired along different axes. Additionally, DTI is a quantitative MRI technique where molecular motion
is derived using tensor-based geometry to estimate the direction and magnitude of diffusion at the voxel level (Basser, P. J. & Pierpaoli, C., 1996; Pierpaoli, C., Jezzard, P., Basser, P. J., Barnett, A., & Di Chiro, G., 1996). Indices of tissue pathology are derived using these tensor-based measurements of diffusion.

Thus, DTI can provide a rotationally invariant estimate of the 3D shape of water diffusion to infer microstructural integrity of the brain’s main communication pathways, the white matter (Mori & Zhang, 2006). There are two commonly used metrics obtained from DTI imaging: fractional anisotropy (FA), which measures directionality of diffusion within a voxel, and mean diffusivity (MD), which measures the average diffusion within a voxel.

FA is an index of the degree of anisotropic diffusion, with a high value indicating high anisotropic diffusion (ranging from 0-1). Low FA values (below 0.3) are believed to reflect conditions where there is compromise in the brain’s white matter structure, causing an increase in free diffusion (equally in all directions) of water in and around the white matter fibres. High FA values (above 0.35) are believed to reflect conditions where bundles of axons are highly ordered and/or myelinated, thus causing a perpendicular barrier to the free diffusion of water (Malloy, P., Correia, S., Stebbins, G., & Laidlaw, D. H., 2007). Alternatively, high MD values are believed to reflect a loss of anisotropic diffusion as indicated by an increase in free diffusion. These DTI metrics have recently been used to examine brain-behaviour relationships in patients with dementia, where distribution patterns of tissue damage have been correlated with performance on various neuropsychological tests (Bozzali, M. et al., 2002).
Although recent studies have demonstrated differences in white matter integrity between AD patients and normal elderly controls, conflicting results indicate the need for more standardized approaches to properly reap the benefits of this relatively new neuroimaging technique. One study examining MD and FA measures obtained from manually placed ROIs of variable size, demonstrated higher MD and lower FA values in AD patients vs. NCs (Bozzali, M. et al., 2002). This decrease in anisotropic diffusion was found in ROIs placed in frontal, temporal and parietal white matter of AD patients; however, no difference was demonstrated in occipital and internal capsule regions. Additionally, a strong correlation was found between MMSE scores and whole brain white matter MD (r=0.92, p<0.001) and FA (r=0.78, P<0.001). Another series of studies using a fibre tract-based approach demonstrated similar results, with abnormally high MD and low FA values found along posterior cingulate fibre tracts of AD patients compared to normal elderly controls (Nakata, Y. et al., 2008). However, in contrast to the Bozzali et al. (2002) study, no correlation was demonstrated between DTI measures and MMSE score (Nakata, Y. et al., 2009). Another recent study replicated Bozzali et al. (2002) results in the frontal white matter in AD; however, conflicting results were found in the occipital region, where significantly lower FA values were also demonstrated (Sjobeck, M. et al., 2010). While differences in methodological approach (ROI-based vs. tract-based) may explain some of these conflicting results, changes in microstructural diffusion has been demonstrated to provide unique and independent pathological information in AD patients, which was previously unobservable with standard macroscopic MRI-derived volumetrics (Canu, E. et al., 2010; Canu, E. et al., 2011).
Combining DTI with macroscopic evaluations of leukoariosis obtained from segmentation of standard PD/T2/FLAIR MRI allows for the quantitative examination of normal appearing white matter (NAWM). Using a unique standardized ROI approach, the LE processing pipeline described in Chapters 2-4 of this thesis allowed for this type of examination of NAWM. Based on a sub-sample of participants used in Chapter 4 of this thesis, a small pilot study was conducted to compare FA differences in NAWM of AD patients and age-matched normal controls and to correlate these regionalized FA values with a numerical working memory task.

The backward digit span (BDS) test is a working memory/executive function task which involves spatial information processing, manipulating/re-ordering, and mental tracking. A recent fMRI study by Hale et al. (2007), examining BDS performance in ADHD patients suggest 3 parietal circuits associated with BDS performance (Hale, T. S., Bookheimer, S., McGough, J. J., Phillips, J. M., & McCracken, J. T., 2007). The bilateral superior parietal regions are especially important in ordering, updating, and manipulating information, particularly spatial or numerical information, in working memory (Dehaene, S., Piazza, M., Pinel, P., & Cohen, L., 2003). Frontal (particularly dorsal lateral) regions are also thought to help mediate these types of processes. Whether white matter microstructural integrity in these regions correlates with BDS performance, has not been examined.

Thus, the current pilot project used fractional anisotropy as a quantitative tensor-based diffusion estimate to examine the structural integrity of normal appearing white matter to determine if AD pathology may be associated with microstructural changes.
which are not observable on standard structural MRI. Regional changes in diffusion were correlated with an executive functioning task.

Participants were obtained from a sub-sample of participants studied in Chapter 4 of this thesis, which included normal elderly controls (n=14) and patients meeting NINCDS-ADRDA criteria for probable AD, and DSM-IV criteria for dementia (n=15). In addition to the standard structural scanning acquisitions (T1, PD/T2), DTI scanning parameters (12 tensor directions) were also obtained using a 1.5T GE scanner. Patients varied in degree of white matter subcortical hyperintensities (WMSH). All patients underwent neurobehavioural and standardized neurological assessment (including BDS), and neuroimaging.

Each set of MRIs were coregistered using the Automated Image Registration (AIR 5) package from UCLA, California. Using the semi-automated brain region extraction (SABRE) procedure, each brain was individually segmented to obtain 26 brain regions with each voxel classified into 4 tissue classes: GM, WM, CSF and vCSF (Dade, L. A. et al., 2004; Kovacevic, N. et al., 2002). The LE processing pipeline was performed to segment SH to include this common 5th tissue class seen in the elderly and more frequently in AD as discussed in Chapter 4 (Ramirez, J. et al., 2011). DTI Studio software from John Hopkins University was used to generate fractional anisotropy (FA) maps of each individual DTI. Gray matter, CSF, vCSF and WMSH were removed from the segmented image, leaving a SABRE regionalized white matter mask, which was applied to the eddy current corrected FA map after coregistration to the DTI image. SABRE regionalized FA measures were obtained using the ANALYZE software package. See Figure 5.1.
A Mann-Whitney non-parametric U test was performed comparing the SABRE regionalized mean FA values from the normal appearing white matter (NAWM) of AD patients and NC. Whole brain FA of NAWM was significantly lower in AD vs. NC (AD=0.33±0.03 vs. NC=0.35±.02, p<.02).

SABRE regionalized FA values using Spearman’s test, revealed significant correlations between BDS scores and FA in the left superior parietal (r=.44, p=.036), left inferior parietal (r=.45, p=.034), and right superior parietal (r=.42, p=.044) SABRE regions. A scatterplot combining FA from all 4 parietal regions (left+right+superior+inferior) is shown in Figure 5.2.
Figure 5.2: FA-BDS Scatterplot. A scatterplot showing mean fractional anisotropy (FA) of bilateral inferior and superior parietal regions against Backward Digit Span (BDS) scores revealing a moderate positive correlation (Spearman’s r=.45, p=.03).

The results of this pilot study suggest that Alzheimer’s pathology is associated with compromised microstructural integrity of whole-brain normal-appearing white matter. Moreover, this pilot study revealed that poorer performance on this simple working memory task was associated with compromised microstructural integrity of normal appearing white matter in the parietal regions. These results suggest that the compromised parietal circuitry, as indicated by lower mean FA in this region, may be important for information transfer in a numerical working memory task.

Previous functional studies in the fMRI literature show bilateral superior parietal (BA7) activation during performance of various working memory and executive functioning tasks and particularly those involving ordering of spatial elements or numbers such as the BDS. In addition, the left inferior parietal region appears to be
important when verbal processing is also required (Dehaene, S., Piazza, M., Pinel, P., & Cohen, L., 2003), which may explain why we found FA to be associated with BDS scores in this region as well. These parietal regions are believed to work with dorsal lateral frontal regions also involved in manipulating/ordering information in the 3 networks thought to mediate number-related processes (Dehaene, S., Piazza, M., Pinel, P., & Cohen, L., 2003).

White matter is the brain’s primary circuitry mediating efficient information transfer. The current study indicated compromised microstructural integrity of AD patients’ white matter, is related to deficits in the putative circuitry required for normal working memory functioning. This compromise may be exacerbated in the presence of vascular injury represented by WMSH observed on PD/T2-weighted and FLAIR MRI.

A post-hoc analysis indicated significantly greater volumes of WMSH in the parietal region of AD (2649mm$^3$) vs. NC (739mm$^3$), p<0.01. These results further support the concept that SH pathologically influences the microstructural integrity of surrounding normal appearing white matter – possibly in a graded manner (Maillard, P. et al., 2011).

While limited by its sample size, this pilot study demonstrated the LE pipeline’s versatility, showing how it can be combined with additional imaging techniques such as DTI. The combined information from these 2 techniques may provide further insight into the deficits associated with WMSH and the white matter circuitry required for normal brain network functioning.

Recent work examining DTI’s utility for examining MCI as a prodromal precursor to AD have shown some promising results, suggesting that regional microstructural
changes in diffusion may indicate risk for conversion to dementia (Kantarci, K. et al., 2005; Pievani, M. et al., 2010; Scola, E. et al., 2010; Stebbins, G. T. & Murphy, C. M., 2009). Additionally, limitations from manually placed and standardized ROI-based techniques to examine DTI have led to more sophisticated tract-based approaches which will be discussed in the following section.

5.3 Tract-based Spatial Statistics (TBSS)

While the combination of DTI and the LE pipeline’s ROI-based method may provide information from traditional neuroanatomical regions, novel techniques for multi-subject DTI analyses are currently being developed to examine differences in anisotropic diffusion along specific white matter tracts. Voxel-based morphometry (VBM) is a commonly used voxel-wise statistical analysis approach applied to structural imaging to correlate brain regions with different covariates of interest, such as group comparisons and cognitive performance scores. The VBM analysis technique involves performing spatial normalization, GM segmentation, and spatial smoothing operations on each individual image in the analysis and can be applied to a variety of structural imaging (Ashburner, J. & Friston, K. J., 2000). However, recent criticisms of VBM, particularly as applied to FA data analysis, include: the registration accuracy of FA maps to a standard space; and the variability of results that can be produced during the spatial smoothing operation. This criticism led to the recent development of tract-based spatial statistics (TBSS) for multi-subject analysis of FA, which addresses the potential for misaligned images during registration and the lack of standardization regarding the extent to which the smoothing operation is applied when using VBM for DTI analysis (Smith, S. M. et al., 2006; Smith, S. M. et al., 2007).
In contrast to VBM, the TBSS approach does not require precise alignment of FA images to a standard template; it only requires an approximate non-linear registration that is followed by projection of the data to an alignment-invariant tract – referred to as “the mean FA skeleton” (Smith, S. M. et al., 2006). The skeleton is generated algorithmically using center-of-gravity, voxel search, and smoothing operations applied to the voxel values obtained from a mean FA image. Essentially, the skeleton is an estimate for the locations of all tracts common to all subjects in the study. After alignment and skeletonisation, each individual subject’s FA image is projected onto the mean FA skeleton, where paired voxel-wise statistics can be performed along the same skeleton.

The TBSS analysis toolset is available online as a free software download via the FSL library (http://www.fmrib.ox.ac.uk/fsl/). This technique was recently applied in our laboratory to a small sample of patients with primary progressive aphasia (Schwindt, G. C. et al., 2011) and is currently being applied to a subset of AD patients used in this thesis to examine correlations between regional volumetrics, cognitive performance, and TBSS-derived anisotropy. The TBSS approach is ideal for studying a dementia population, where the precision of warping registration techniques is poor due to the brain atrophy and ventricular enlargement that is commonly observed in AD pathology. Furthermore, serial DTI analyzed using the TBSS approach may provide further insight into the microstructural progression of white matter disease in this population.

5.4 Anisotropy Surrounding and Within Hyperintensities

Compromised microstructural integrity of the NAWM surrounding SH may also reveal that hyperintensities macroscopically observed on standard structural MRI only
indicate the “tip of the iceberg”, as subtle microstructural changes are occurring in the white matter penumbra surrounding the SH region. A recent study performed on a large set of AD and MCI patients provides DTI data that supports the penumbra hypothesis, demonstrating a decrease in mean FA of 0.012 (p<0.001) from neighboring NAWM voxels as their spatial location approaches SH labeled voxels (Maillard, P. et al., 2011).

In addition to examining the penumbra of NAWM surrounding SH, DTI could also be used to examine the anisotropy within SH. A novel technique is currently being developed to examine heterogeneity found within different SH sub-types in a subset of the sample used in this thesis. Using the LE pipeline, SH sub-types are segmented and spatially registered to the FA image. Voxel-wise FA values within the SH subtype is entered into a k-means cluster analysis with a Euclidean distance measure for 3 clusters analysis (high, medium, low). See Figure 6.3 for an example of data obtained from a single test subject.

**Figure 5.3: Lesion Voxel Cluster Analysis.** Image on the left shows a single axial slice of an FA image with the pvSH voxels labeled using a lesion voxel cluster analysis. Graph on the right displays the range of FA values within the pvSH lesions. Red indicates low lesion FA, green shows medium lesion FA and blue indicates high lesion FA. The y-axis indicates the FA values, the x-axis shows the number of voxels. Note the graph shows the whole range of FA values within pvSH in all 3-dimensions.
Recent advances in DTI-based tractography show promise (Basser, P. J., Pajevic, S., Pierpaoli, C., Duda, J., & Aldroubi, A., 2000). Innovative approaches such as TBSS (Smith, S. M. et al., 2006) and neighborhood voxel-based analysis within and surrounding (Maillard, P. et al., 2011) leukoariosis may provide additional insight from pre-existing datasets. Given the LE processing pipeline’s component-based approach, innovations such as these can be integrated into the pipeline with some minor modifications, making the LE pipeline a good platform for future advances in neuroimaging techniques.

5.5 Ventricular enlargement

It has been suggested that measures of brain tissue atrophy rates derived from structural MRI may be used as surrogate markers for disease progression in observational studies of aging, prodromal or clinical stages of dementia, or potentially for disease-modifying therapeutic trials in mild cognitive impairment (MCI) and AD (Fox, N. C., Cousens, S., Scahill, R., Harvey, R. J., & Rossor, M. N., 2000; Jack, C. R., Jr. et al., 2003; Jack, C. R., Jr. et al., 2004). Ventricular cerebrospinal fluid (vCSF) volume is one commonly used metric to assess brain tissue atrophy, where ventricular expansion rates have been associated with aging, sex, education, diabetes, hypertension, and poorer performance on tests of global cognitive function, memory, executive control functions (Adak, S. et al., 2004; Breteler, M. M. et al., 1994; Carmichael, O. T. et al., 2007b; Hua, X. et al., 2010; Scahill, R. I. et al., 2003).

In cross-sectional studies examining aging and dementia, AD patients have been observed to have significantly larger ventricles relative to normal controls, with MCI
patients in between (Apostolova, L. G. et al., 2012). Longitudinally, AD patients and MCI patients who eventually convert to AD, generally show accelerated ventricular expansion rates when compared to non-demented elderly and stable MCI (Carmichael, O. T. et al., 2007a; Evans, M. C. et al., 2010; Giesel, F. L. et al., 2006; Nestor, S. M. et al., 2008; Schott, J. M. et al., 2005). Moreover, higher ventricular expansion rates are associated with increased hazard of conversion from MCI to AD and baseline vCSF volumes may be used to predict future cognitive decline (Carmichael, O. T. et al., 2007b; Fleisher, A. S. et al., 2008; Schott, J. M. et al., 2010).

Additionally, variations in the apolipoprotein E (ApoE) genotype may influence ventricular expansion rates. Several studies have shown variations in ApoE genotypes have differential responses to treatment of AD and are believed to be associated with different rates of brain tissue atrophy (Bizzarro, A. et al., 2005; Chou, Y. Y. et al., 2010; Nestor, S. M. et al., 2008; Okonkwo, O. C. et al., 2010). More specifically, carriers of the ApoE ε4 allele have been demonstrated to have a genetic risk factor for sporadic AD (Blesa, R. et al., 1996), are more likely to have increased amyloid neuritic plaque deposition (Nagy, Z. et al., 1995), and are more likely to have an accelerated rate of ventricular expansion and brain atrophy (Schmechel, D. E. et al., 1993; Wahlund, L. O., Julin, P., Lannfelt, L., Lindqvist, J., & Svensson, L., 1999).

Thus, it can be hypothesized that AD patients will exhibit greater vCSF volume changes as compared to normal elderly. Moreover, as individuals who are ApoE ε4 positive are at greater risk for brain atrophy, it was hypothesized that patients with one or more copies of the ε4 allele would have greater ventricular expansion rates compared to those without.
Furthermore, the rate of vCSF volume change over time, estimated from serial antemortem MRI, has been shown to correlate with post-mortem measures of senile plaque and neurofibrillary tangle severity, lending further support that the rate of vCSF volume enlargement can be used as a valid surrogate biomarker of pathologic progression of AD with its utility to examine response to disease-modifying investigational treatment in clinical trials (Fox, N. C. et al., 2005; Silbert, L. C. et al., 2003).

In summary, while controlling for potential confounds, ventricular expansion rates and baseline vCSF volumes have been associated with disease progression in dementia studies, providing an objective, quantitative surrogate biomarker in longitudinal studies with serial MRI (Nestor, S. M. et al., 2008).

A brief pilot study examining the rate of vCSF expansion was conducted on a sub-sample of participants used in Chapter 4 of this thesis who have serial MRI. This pilot was used as a preliminary analysis to establish proof of concept for future longitudinal projects which will use the LE pipeline for serial volumetrics as potential biomarkers for disease progression and for response to treatment in clinical trials. Additionally, results from the short-term scan-rescan study described in Chapter 2 of this thesis were used to establish technique-related error guidelines when using the LE pipeline for longitudinal research (Ramirez, J., Scott, C. J., & Black, S. E., 2012).

Participants included patients meeting NINCDS- ADRDA criteria for probable AD, and DSM-IV criteria for dementia (n=108, female=55) and normal controls (n=43, female=24). Mean age at baseline was as follows: AD=70.7±9.5, NC=69.7±10.5 (p=0.41). Participants were stroke-free but had varying degrees of SH volumes (i.e.,
individuals with large amounts of SH were not selectively removed from the sample).
All patients underwent neurobehavioural assessment, standardized neurological assessment, neuroimaging, and ApoE genotyping. Mean inter-scan interval (ISI) was 1.2±0.12 yrs (range=1.0-1.9 yrs). See Table 5.1 for demographic summaries.

Each set of MRIs were obtained using the same 1.5T GE scanner. Images were then segmented using a simplified version of the LE imaging pipeline, previously described in Chapters 2-4 of this thesis and can be found in published format elsewhere (Dade, L. A. et al., 2004; Kovacevic, N. et al., 2002; Ramirez, J. et al., 2011). The serial MRI segmentation procedure described in Chapter 3 of this thesis provides a framework for this analysis, which used baseline Total Intracranial Capacity (TIC) as this would not change over time and reduced one source of measurement variance. This has been shown to be a highly reliable technique, ICC=0.9998 for vCSF volumetrics (Ramirez, J. et al., 2011; Ramirez, J., Scott, C. J., & Black, S. E., 2012). Ventricular delineation was performed in an individualized manner using the semi-automated 3D segmentation procedure from SABRE, where CSF voxels were automatically segmented and then manually reassigned to vCSF by trained raters, see Figure 5.4 (Dade, L. A. et al., 2004; Kovacevic, N. et al., 2002).

In the short-term scan-rescan reliability test described in Chapter 3, a mean absolute volume difference of 682mm$^3$ was demonstrated for vCSF when the LE pipeline was applied to normal elderly (Ramirez, J., Scott, C. J., & Black, S. E., 2012). This difference can be attributed to technique-related and random error, which translates to a 2.6% expected measurement error when expressed as a percentage relative to baseline measures. Given these previous results, a safety margin was set
where any vCSF change below 0.7cc (or <3%) of baseline was considered within the range of measurement error and thus considered as no significant change using a 95% confidence interval for analyses. Change in vCSF volume was calculated as a percentage relative to baseline, and then expressed as vCSF delta rate over a 12 month period (i.e., yearly rate of ventricular expansion).

An analysis of covariance (ANCOVA) was used to analyze both raw volumetric changes (vCSF Delta) and annualized rate of volumetric change expressed as a percentage of baseline (vCSF Delta Rate), with age and sex entered as covariates.

Ventricular expansion rate over an estimated one year period was greater in AD vs. NC (p<0.001), with the vCSF Delta and vCSF Delta Rates in AD exceeding

![Figure 5.4: 3D Volume render of ventricular CSF. Top row: view from left and right sides, Middle row: view from front and back, Bottom row: view from top and bottom.](image)
technique-related error overestimates of 3% Delta Rate and 0.7cc volumetric difference.

Mean vCSF delta rate was 10% for AD and 4% for NC, and mean raw volumetric differences were 5.2cc for AD and 2.0cc for NC. While Age and Sex were entered as covariates, the F-test results showed that they did not appear to have a significant influence on vCSF Delta (Age: p=0.25, Sex: p=.251) in this sample. See Table 5.1 for a summary of these results.

Table 5.1: Summary of demographics and vCSF changes comparing AD and NC.

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>NC</th>
<th>p-value</th>
<th>Cohen's d</th>
<th>Effect size r</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>108</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISI (yrs)</td>
<td>1.2</td>
<td>1.3 ±0.25</td>
<td>&lt; 0.001</td>
<td>0.9</td>
<td>0.41</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>53/55</td>
<td>19/24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (Baseline)</td>
<td>70.7 ±9.5</td>
<td>69.7 ±10.5</td>
<td>&lt; 0.001</td>
<td>0.9</td>
<td>0.41</td>
</tr>
<tr>
<td>ApoE E4 +/-</td>
<td>75/33</td>
<td>n/a</td>
<td>&lt; 0.001</td>
<td>0.9</td>
<td>0.41</td>
</tr>
<tr>
<td>vCSF1 (cc)</td>
<td>50.7</td>
<td>35.1</td>
<td>&lt; 0.001</td>
<td>0.9</td>
<td>0.41</td>
</tr>
<tr>
<td>vCSF2 (cc)</td>
<td>±24.9</td>
<td>±17.7</td>
<td>&lt; 0.001</td>
<td>0.9</td>
<td>0.41</td>
</tr>
<tr>
<td>vCSF Delta (cc)</td>
<td>±26.2</td>
<td>±18.9</td>
<td>&lt; 0.001</td>
<td>0.9</td>
<td>0.41</td>
</tr>
<tr>
<td>vCSF Delta Rate</td>
<td>10.0%</td>
<td>3.7%</td>
<td>&lt; 0.001</td>
<td>0.9</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Additionally, patients who tested as positive carriers of one or more copies of the ApoE e4 allele (n=75) displayed a trend for greater yearly ventricular expansion rate compared to non-carriers (p=0.07). Mean vCSF delta rate was 11% for e4 carriers compared to 7% without. See Table 5.2 for a summary of these results.

The results from this pilot study demonstrate that ventricular enlargement is a quantifiable measure that can be reliably measured in the elderly and AD over the
course of 12 months. While ventricles expand in normal elderly individuals, the rate of change is accelerated in patients with AD and appears to be further exacerbated in AD patients who are ε4 positive carriers. These results are congruent with previous studies reported in the literature (Bradley, K. M. et al., 2002; Carmichael, O. T. et al., 2007a; Evans, M. C. et al., 2010; Giesel, F. L. et al., 2006; Nestor, S. M. et al., 2008; Schott, J. M. et al., 2005), and are thus promising results for the use of the LE pipeline when planning future longitudinal studies requiring a quantitative and valid surrogate biomarker for pathologic progression of AD in clinical trials (Fox, N. C. et al., 2005; Silbert, L. C. et al., 2003). This is of note since the current AD sample is more representative of patients attending a tertiary clinic as it included a wider range of severity of subcortical white matter disease than is typical in studies such as the Alzheimer Disease Neuroimaging Initiative or in current clinical trials of AD.

Table 5.2: Ventricular volume change comparing ApoE ε4 positive vs. ε4 negative patients with AD.

<table>
<thead>
<tr>
<th></th>
<th>ApoE ε4+</th>
<th>ApoE ε4-</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>75</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>ISI (yrs)</td>
<td>1.2±0.12</td>
<td>1.2±0.13</td>
<td></td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>37/38</td>
<td>16/17</td>
<td></td>
</tr>
<tr>
<td>Age (Baseline)</td>
<td>70.1±8.7</td>
<td>72.2±11.2</td>
<td></td>
</tr>
<tr>
<td>vCSF1 (cc)</td>
<td>47.7±20.3</td>
<td>57.0±32.8</td>
<td></td>
</tr>
<tr>
<td>vCSF2 (cc)</td>
<td>53.1±21.8</td>
<td>61.5±34.1</td>
<td></td>
</tr>
<tr>
<td>vCSF Delta (cc)</td>
<td>5.4±4.7</td>
<td>4.5±3.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>vCSF Delta Rate</td>
<td>11%</td>
<td>7%</td>
<td>p=0.07, n.s.</td>
</tr>
</tbody>
</table>

While the normal elderly group and the AD patients were matched for age, future investigations using ventricular expansion rates should include education as a covariate
in the analysis, as some studies have demonstrated a relationship with ventricular atrophy and education (Carmichael, O. T. et al., 2007a; Carmichael, O. T. et al., 2007b). Also, the relationship with ventricular expansion and signs of vasculopathy in the periventricular region should be examined to further explore potential additive effects of the co-pathology, which can be severe in 20% of the normal population. Investigations examining associations with measures of cognition and memory would provide additional insights into the potential role of confluent white matter disease in brain atrophy progression in all tissue compartments as well as ventricular expansion rates.

Despite these and other findings, it is important to note that although ventricle volumes and ventricular expansion rates have been associated with AD pathology (Apostolova, L. G. et al., 2012), conversion from MCI to dementia (Carmichael, O. T. et al., 2007a), and decline in everyday function (Okonkwo, O. C. et al., 2010), the neurobiological mechanisms that underlie these associations remain unclear.

### 5.6 Sex Differences

Sex differences examining risk for developing dementia have been examined in various population-based studies with mixed results. When determining incidence rates and risk profiles for dementia, various population-based studies show mixed results, with some studies suggesting that women are at greater risk for developing dementia (Di Carlo, A. et al., 2002; Fratiglioni, L. et al., 1997; Letenneur, L. et al., 1999; Miech, R. A. et al., 2002; Ott, A., Breteler, M. M., van Harskamp, F., Stijnen, T., & Hofman, A., 1998), while others suggest no difference between the sexes (Ganguli, M., Dodge, H. H., Chen, P., Belle, S., & DeKosky, S. T., 2000; Hebert, L. E., Scherr, P. A., McCann, J.,

In a post-mortem histopathological study which directly examined neuritic plaques and neurofibrillary tangles from a population study of 141 older Catholic clergy members, it was found that women had more global AD pathology compared to men (Barnes, L. L. et al., 2005). Additionally, when examining the association with AD pathology and the clinical expression of dementia, it was determined that AD pathology was more likely to be expressed in women. More specifically, women had a 20 fold increase in the odds of expressing dementia compared to a 3 fold increase in men, with each additional unit of AD pathology. These results suggest that the clinical manifestation of AD is stronger in women.

When examining the contribution of vascular risk, a different story unfolds regarding sex differences. A recent report from the Framingham Offspring Study, which examined neuropsychological test performance and cerebrovascular disease (as defined by volume of SH plus cerebral infarcts on MRI) in 2085 non-demented elderly participants, showed a positive relationship between performance on executive function and cerebrovascular disease in men but not in women (Albert, M. et al., 2010). Interestingly, the finding only held true for men (but not for women) who were at risk for stroke, which was determined using the Framingham Stroke Risk Profile score (Wolf, P. A., D'Agostino, R. B., Belanger, A. J., & Kannel, W. B., 1991). Apart from this difference, the MRI measures for whole brain atrophy and “subclinical” markers of vascular disease (i.e., hyperintensities) showed no difference between the sexes.
A small pilot study was performed to examine sex differences in a sub-sample of AD and NC used in Chapter 4 of this thesis. Comparisons of whole brain, GM and WM brain tissue atrophy, and signs of vascular injury as indicated by various SH sub-types was examined using volumetrics derived using the LE processing pipeline described in this thesis and previously published elsewhere (Ramirez, J. et al., 2011).

Participants included patients meeting NINCDS- ADRDA criteria for probable/possible AD, and DSM-IV criteria for dementia AD (m/f: n=88/88) and normal controls (m/f: n=35/42), sampled from the Sunnybrook Dementia Study. Age and education details are shown in Table 5.3. Participants were stroke free but had varying degrees of SH volumes (i.e., individuals with large amounts of SH were not selectively removed from the sample). All patients underwent neurobehavioural assessment, standardized neurological assessment and neuroimaging.

<table>
<thead>
<tr>
<th>Dx</th>
<th>Sex</th>
<th>n</th>
<th>Age</th>
<th>Education</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Men</td>
<td>88</td>
<td>72.8 ± 9.6</td>
<td>13.0 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>88</td>
<td>74.6 ± 8.5</td>
<td>13.2 ± 3.4</td>
</tr>
<tr>
<td>NC</td>
<td>Men</td>
<td>35</td>
<td>71.7 ± 8.5</td>
<td>16.0 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>42</td>
<td>68.7 ± 8.1</td>
<td>15.3 ± 2.8</td>
</tr>
</tbody>
</table>

Three image sets were acquired for each pair of scans: a T1-weighted, an interleaved proton-density (PD) and a T2-weighted image. Each individual's MRIs were segmented using the LE imaging pipeline, previously described in Chapters 2-4 of this thesis which can also be found in published format (Ramirez, J. et al., 2011). In general, the LE process can provide: parenchymal volumes, total intracranial capacity
(TIC), whole brain GM and WM, pvSH and dwSH, and lacunar volumetrics for comparison.

A multivariate analysis of covariance (MANCOVA) was used to analyze the brain atrophy measures, with age and education entered as covariates. Volumetrics were head size corrected using TIC. Since SH volumes were non-normally distributed, a non-parametric Mann-Whitney U test was used to compare the various SH subtypes (pvSH, dwSH, and lacunes).

As expected, men generally had a larger total intracranial capacity (p<0.001). Using head size corrected data, a significant difference was found with NC males showing more GM (p<0.0001), but no difference in WM volume (p=0.12, n.s.). Although head size corrected data was used for this analysis, this difference can be illustrated with mean raw volumetrics, showing NC male GM=581.5cc vs. NC female GM=546.7cc. The AD patients showed a similar pattern, with no significant difference in WM (p=0.86, n.s.) but statistically significant differences in volumes of GM (p<0.01), with mean raw volumetrics showing AD male GM=525.7cc vs. AD female GM=479.0cc.

Results from the non-parametric analysis (Mann-Whitney U) revealed a different pattern of sex difference for AD and NC. In NC, women had less pvSH (p<.0001) and fewer lacunes (p<.0001) than men but similar dwSH volumes. However, in AD, women had more pvSH (p<.05), and more dwSH (p<.05) when compared to men with AD but similar lacunar volumes.

The preliminary results from this pilot study suggest a sex difference in distribution of leukoaraiosis with AD pathology. Normal elderly women showed less evidence of vascular injury in the periventricular region and less evidence of silent brain
infarcts. However, women with dementia showed more signs of leukoariosis than their male counterparts. These results are meant to be exploratory and hypothesis generating for future studies examining sex differences. One possibility is that women with AD may harbor more small vessel co-pathology, which could be a further driver of amyloid cascade.

A recent study examining 3 yr progression rates for dwSH and pvSH found that while leukoariosis in the periventricular region had similar rates of progression for normal elderly men and women, the progression rate for dwSH was greater in women (van den Heuvel, D. M. et al., 2004). More specifically, women were reported to have double the amount of dwSH volumes compared to men after a 3 yr follow-up period. Future longitudinal examinations should be considered to determine if the results from this preliminary pilot work demonstrate similar sex differences when examining signs of vascular pathology in both demented and non-demented participants.

5.7 Cortical thickness

In addition to volumetric studies, cortical thickness has also been used to index atrophy in the aging brain, with various studies demonstrating age-related cortical thinning (Dale, A. M., Fischl, B., & Sereno, M. I., 1999). In one study examining a wide age-range of 106 participants (18-93yrs), the cortical rim was shown to decrease by 0.01mm/decade, with men having an overall thicker cortex, although this disappeared with head size correction (Salat, D. H. et al., 2004). In this study, loss of GM volume was greatest in the inferior prefrontal, precentral, and the supramarginal regions. Interestingly, cortical GM volume within the temporal lobe was relatively preserved in AD compared to NC.
A recent study reported that cortical thickness distinguished AD from normal elderly controls, with an overall accuracy of 75%, sensitivity of 79%, and specificity of 71%. However, cortical thickness in the parahippocampal gyrus provided the highest accuracy (94%) (Lerch, J. P. et al., 2008). Increased availability of automated cortical thickness estimation software will allow future studies to combine this measure with other techniques for structural analysis of the aging brain. A precaution however, is that these techniques may not work well in patients with significant amounts of white matter disease and consequently, was not applied to the sample in this thesis. This methodological issue may be addressed in future developments of cortical thickness approaches and could easily be applied to this sample population, if the technique was more robust to age related white matter changes.

5.8 Cerebrovascular reactivity (CVR)

Recent imaging innovations examining cerebrovascular reactivity (CVR) show promising advances towards the understanding of the brain’s vascular functioning and may contribute to our understanding of SH (Conklin, J. et al., 2010; Han, J. S. et al., 2011a; Han, J. S. et al., 2011b; Mandell, D. M. et al., 2008b; Mandell, D. M. et al., 2008a). CVR refers to the ability for blood vessels to dilate in response to a vasodilatory stimulus. This technique makes use of the blood vessels’ ability to control blood flow locally through vasodilation via changes in the smooth muscle tone. Through this vasodilatory mechanism, autoregulation of cerebral blood flow occurs, allowing the brain to maintain a relatively stable blood flow across a range of blood pressures. This compensatory mechanism allows blood vessels to lower their resistance to flow in
circumstances where blood pressure decreases or when a vasodilatory stimulus, such as CO2, is introduced (Han, J. S. et al., 2011a).

However, autoregulation of cerebral blood flow has a finite capacity (Derdeyn, C. P. et al., 2002). Areas of the brain where this compensatory mechanism is exhausted leads to reduced blood flow. Additionally, a steal phenomenon can occur, whereby blood flow is redirected to areas of the brain where the compensatory mechanism is normally functioning, further reducing blood flow to the exhausted region. Using blood oxygen level-dependent (BOLD) and/or arterial spin labeling (ASL) MRI to measure cerebral blood flow, and a breathing apparatus to control inhalation of CO2, the blood flow response can be measured to examine CVR (Mikulis, D. J. et al., 2005; Vesely, A. et al., 2001).

BOLD and more recently, ASL, are both neuroimaging techniques that can be used to assess cerebral blood flow in CVR experiments. BOLD contrast MRI makes use of the hemodynamic response, associated with neuronal activation, by measuring magnetic signal differences in oxyhemoglobin and deoxyhemoglobin (Kwong, K. K. et al., 1992; Roberts, T. P. & Mikulis, D., 2007; Rosen, B. R. et al., 1993). While BOLD MRI is typically used in functional MRI (fMRI) studies, in the case of CVR, BOLD MRI is used as an indirect measure to estimate changes in cerebral blood flow. ASL is an MRI technique which uses magnetically labeled arterial water as a tracer to measure cerebral blood flow (Detre, J. A. et al., 1994; Macintosh, B. J. et al., 2010). Both imaging techniques may be used to examine CVR; however, the relatively newer and less established ASL technique requires further testing for proper implementation in CVR studies.
A CVR study performed on healthy volunteers using an automated gas sequencer to administer high and low end-tidal partial pressure of carbon dioxide (PETCO2) found a steal phenomenon in regions of the brain typically associated with SH in the elderly (Mandell, D. M. et al., 2008a). Following this framework, it can be hypothesized that the observable SH or white matter injury may be a result of progressive exhaustion or poorer autoregulatory functioning of the blood vessels in those regions of the brain. This can be examined through spatial mapping of induced autoregulation using CO2 and BOLD MRI, whereby areas with poorer CVR are predicted to develop SH in follow-up scans. Work on this hypothesis is currently underway.

5.9 Future Directions Summary

Advanced imaging techniques such as DTI can be combined with the LE pipeline to further our understanding of leukoariosis. DTI may be used to examine white matter changes at the microstructural level and also allow us to make predictions based on compromised structural integrity, possibly along specific white matter tracts. A small pilot study (section 5.2) demonstrated how the LE pipeline may be used to segment normal appearing white matter and create individualized SABRE brain regions. Despite the small sample size, results from this pilot study revealed a significant association with performance on a working memory task and white matter integrity in the parietal region. Studies which will apply TBSS (section 5.3) and a novel method to examine the penumbra surrounding SH (section 5.4) are currently underway, which may provide additional insight regarding age-related white matter changes and dementia.
Additionally, a small pilot study examining yearly ventricular expansion rates was performed on a sub-sample of participants with longitudinal data, to demonstrate the LE pipeline’s 3D ventricular segmentation as applied to serial MRI measures (section 5.5). This study was an extension of the short-term scan-rescan study described in Chapter 3 of this thesis, which established a metric for technique-related error when planning longitudinal studies using the LE procedure. Results from this pilot work demonstrated how LE’s vCSF volumetrics can be used to determine ventricular expansion rates to discriminate between AD and NC, with AD’s showing a significantly greater yearly expansion rate (10% vs. 4%). Moreover, AD patients with one or more copies of the ApoE ε4 allele exhibited greater ventricular expansion compared to those without the ε4 allele (11% vs. 7%). These promising results indicate the potential for the LE pipeline to be used to generate ventricular expansion rates for longitudinal studies and possibly for application in clinical trials as a surrogate marker for pathological changes relating to brain atrophy.

A brief analysis examining sex differences was also described (section 5.6), which suggest a potential greater susceptibility to leukoariosis in women with AD. These results may serve as preliminary analyses for future work, which will examine potential sex differences, as these surrogate markers for subcortical vasculopathy progress longitudinally in normal elderly and patients with dementia.

Finally, a brief review of cortical thickness (section 5.7) and CVR (section 5.8) provide some additional direction for future neuroimaging techniques which may be combined with the LE pipeline procedure. While cortical thickness may allow for regional atrophy measures, future work is required to enable this technique to be used
in participants with significant leukoariosis. The brief review of CVR showed how this novel neuroimaging technique may be used to assess areas in the brain with poorer hemodynamic autoregulation and predict areas more vulnerable to leukoariosis and white matter injury.

These studies, which stem from this thesis work, are currently in the process of development. This future research will not only further our understanding of vascular related injury in the brain, it may also assist in directing therapeutic interventions focused on reducing the deleterious effects of mixed subcortical vascular and Alzheimer’s dementia in our aging population.
Chapter 6: Thesis Discussion
6.1 General Summary

Despite recent advances in segmentation techniques and the plethora of readily available free online segmentation software packages for MRI post-processing, the LE processing pipeline described in previous chapters remains a competitive option. The pipeline was specifically optimized for examining an aging and dementia population and provides data at an individual level, allowing researchers to examine single special cases as well as clinical population groups. The LE pipeline provides a comprehensive volumetric profile, which includes regionalized volumetrics for several lesion sub-types and brain tissues, including subarachnoid and ventricular CSF. The technique was systematically validated for inter-rater, intra-rater, inter-method, and scan-rescan reliability and demonstrated excellent results in all tests. Overall, the LE processing pipeline is a versatile and comprehensive tool developed to examine regional and whole brain tissue atrophy and various SH sub-types.

In contrast to other volumetric techniques, LE is more robust to some of the typical neuroimaging issues when examining aging and demented populations. Using a localized histogram approach for basic GM, WM, and CSF segmentation, it is less sensitive to scanner inhomogeneities, age-related issues, and pathology related changes that influence MR image contrast. LE also implements a complex tri-feature segmentation approach, making use of T1, T2 and PD intensity information, allowing for a more accurate brain extraction relative to single feature based approaches such as FreeSurfer and FSL’s BET. Additionally, the tri-feature approach makes LE robust to global atrophy, large variations in ventricle size, and the presence of leukoariosis, allowing for a more inclusive sample set; thus limiting potential subject attrition due to
segmentation faults. Finally, the SH segmentation combines a complex series of intensity cut-off, artificial intelligence, 3D connectivity, and template-matching algorithms, which allow for a reliable and highly reproducible SH sub-type quantification. Thus, the LE technique was developed to systematically address many of the neuroimaging issues commonly associated when developing an MRI-derived volumetrics technique to examine dementia and aging populations.

When applied to a cross-sectional sample of AD patients and normal elderly controls, it was demonstrated that SH location, sub-type, and volume were associated with executive function, mental processing speed, and memory. Specifically, a greater volume of SH in the medial middle frontal region was associated with poorer performance on an executive functioning task, a greater volume of pvSH was associated with longer mental processing times, and a greater volume of SH in the left posterior temporal was associated with poorer performance on a verbal memory task. In summary, these results support the hypothesis that SH location, volume and sub-type may be differentially associated with performance on various cognitive tasks and demonstrate some of the capabilities of the pipeline to examine other brain-behavior research questions.

6.2 Subcortical hyperintensities

It is apparent that SH increase with age, especially with common vascular risk factors (eg., hypertension, diabetes, hypercholesterolemia) (De Leeuw, F. E. et al., 2001; Liao, D. et al., 1996; Sachdev, P., Chen, X., & Wen, W., 2008). The Cardiovascular Health Study reported that only 4.4% of its 3300 participants were free from white matter lesions, and recent reports indicate they increase risk for dementia,
stroke and other disabilities (Kuller, L. H. et al., 2007; Longstreth, W. T., Jr. et al., 1996; Longstreth, W. T., Jr. et al., 2005). Another study reported only 6.5% of participants aged 63-84 were free from white matter burden as assessed on MRI (Williams, L. R. et al., 2010). The distribution of SH are varied, although one study reports more rapid age-related increases of SH in frontal regions compared to temporal and parietal lobes (Jernigan, T. L. et al., 2001). These white matter changes appear to be highly inheritable (Atwood, L. D. et al., 2004).

6.3 Subcortical hyperintensities & Cognition: Clinical Importance?

SH may contribute to cognitive decline, especially executive dysfunction in patients with AD, though this may be stage dependent (Decarli, C. et al., 1996; Hsu, Y. Y. et al., 2002; Smith, C. D., Snowdon, D. A., Wang, H., & Markesbery, W. R., 2000; Wen, W., Sachdev, P. S., Li, J. J., Chen, X., & Anstey, K. J., 2008). In normal individuals, the presence of SH and its relationship with cognition appears to vary with age as well. In a recent report from the John Hopkins ABC study on aging, SH burden showed little to no association with cognition in those aged 20-59yrs; however, participants over the age of 60 exhibited steep cognitive declines with increased SH burden (Vannorsdall, T. D., Waldstein, S. R., Kraut, M., Pearlson, G. D., & Schretlen, D. J., 2009). The presence of SH may begin as early as 30yrs, with one large epidemiological study of 428 normal individuals in their forties (44-48yrs) reporting a SH prevalence of 51% (Wen, W., Sachdev, P. S., Li, J. J., Chen, X., & Anstey, K. J., 2008).

The clinical importance of SH location remains unclear. Some studies claim localization effects (Artero, S. et al., 2004; Burton, E. J. et al., 2004), including one study in a dementia population, which showed that hyperintensities in the anteromedial
thalamus correlated with episodic and working memory, and that SH involving cholinergic tracts compromised executive functions (Swartz, R. H. et al., 2002; Swartz, R. H., Sahlas, D. J., & Black, S. E., 2003); while others report executive dysfunction irrespective of location (Reed, B. R. et al., 2004; Tullberg, M. et al., 2004). In this thesis, SH volume in the medial middle frontal was associated with poorer performance on verbal fluency task, while SH volume in left posterior temporal was associated with poorer verbal memory, thus suggesting that SH location may differentially correlate with different cognitive tasks.

6.4 Periventricular vs. Deep White: Relevance?

The periventricular regions appears to be particularly susceptible to white matter changes in AD and aging, where pvSH have been reported in 48-100% of cases (Brun, A. & Englund, E., 1986; Erkinjuntti, T. & Hachinski, V., 1993; Erkinjuntti, T., Ketonen, L., Sulkava, R., Vuorialho, M., & Palo, J., 1987), with moderate to severe degree reported in 33-50% of AD subjects, particularly associated with older age (Erkinjuntti, T. & Hachinski, V., 1993; Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., & Zimmerman, R. A., 1987). The periventricular white matter is relatively oligaemic as shown in a recent cerebral blood flow study (Brickman, A. M. et al., 2009; Holland, C. M. et al., 2008). Not surprisingly, the periventricular region is topographically where the most confluent and highest volumes of white matter hyperintensity are distributed.

M., 1997), using post-mortem MRI to allow precise MRI-pathological correlations. As discussed recently (Andersson, T., 2010; Black, S. E., Gao, F. Q., & Bilbao, J., 2009), arteriolar small vessel disease may not be the only culprit, though clearly arteriolar occlusive disease of the long penetrating arteries would be expected to cause ischemic damage to the white matter around the ventricles that are dependent on this supply.

In addition, however, Moody, Brown and colleagues have described venous collagenosis in the deep medullary veins that drain in toward the ventricles (Moody, D. M., Brown, W. R., Challa, V. R., & Anderson, R. L., 1995). This appears to be an aging phenomenon, worsened by vascular risks such as hypertension or hypotension, which may arise in response to relative hypoperfusion of these deep regions, leading to gradual fibrosis and thickening of the venous walls, sometimes to the point of occlusion. One result is venous leakage and perivenous vasogenic edema, that may explain the confluent hyperintense signal seen around the ventricle in aging adults (Gao, F. Q. et al., 2008). Thus, these neuropathological studies suggest pvSH to be a reflection of some form of venous insufficiency, while dwSH may reflect arteriolar and venular pathology (Black, S. E., Gao, F. Q., & Bilbao, J., 2009; Brown, W. R., Moody, D. M., Challa, V. R., Thore, C. R., & Anstrom, J. A., 2002; Brown, W. R., Moody, D. M., Thore, C. R., Anstrom, J. A., & Challa, V. R., 2009).

While the design of this thesis does not allow for a direct pathological comparison of white matter changes in the periventricular region and deep white, the results do support the hypothesis that hyperintensities in these different regions are associated with deficits in different cognitive domains (Sachdev, P. & Wen, W., 2005). The central question of this debate is, “Should we distinguish between periventricular and deep
white matter hyperintensities?" (Barkhof, F. & Scheltens, P., 2006; Decarli, C., Fletcher, E., Ramey, V., Harvey, D., & Jagust, W. J., 2005; Sachdev, P. & Wen, W., 2005). The results of this thesis suggest a positive response, at least in a dementia population.

In this study, AD patients showed a significantly larger amount of pvSH compared to normal elderly (13.4cc vs. 4.8cc, p<0.001), while dwSH volumes were relatively similar. These results alone suggest a potential difference in the vulnerability of the vasculature within these two regions. AD pathology may increase vulnerability within the periventricular region to these hyperintensities, or alternatively, venous insufficiency as an independent microvascular disease with genetic loading accelerates AD pathology. An association of microbleeds and SH has been reported in a subset of this population (Pettersen, J. A. et al., 2008) and it may be that venous fibrosis conduces to poor clearance along the perivascular pathways and to amyloid deposition (Black, S. E., Gao, F. Q., & Bilbao, J., 2009).

As further support for the importance of distinguishing between dwSH and pvSH, results of this thesis demonstrate that greater volumes of hyperintensities in the periventricular region was associated with a longer time to perform the Trails A task, while hyperintensities in the deep white region showed no association. While the regression results estimate that a 1% increase in pvSH volume predicts a 6.8 increase in time to complete the Trails A test, statistical predictions of this nature should be taken with caution given the cross-sectional design of this study. However, using similar criteria to loosely define pvSH as any hyperintensity touching the ventricle, a recent progression study demonstrated a similar association with normal elderly using time to complete a Stroop task (van den Heuvel, D. M. et al., 2006a). In an earlier publication,
it was demonstrated that after a 3yr followup, elderly women were shown to have accumulated twice the dwSH volume as elderly men (van den Heuvel, D. M. et al., 2004)(van den Heuvel et al., 2004).

One possible explanation for the association with pvSH and mental processing speed points to the anatomical location of the long association white matter tracts (De Groot, J. C. et al., 2000; De Groot, J. C. et al., 2002; van den Heuvel, D. M. et al., 2006a). Effective completion of complex tasks such as the Stroop and Trails A likely require the communication of anterior-posterior cortical areas. Increased time to complete these tasks may be a reflection of disruptions, as indicated by pvSH, along the long association fibres found in the periventricular region. According to these authors, pvSH may affect communication along the long white matter tracts, while dwSH may affect communication between adjacent cortical areas along the so called “U-fibres” of the brain, thus reflecting deficiencies in different cognitive domains (De Groot, J. C. et al., 2002; van den Heuvel, D. M. et al., 2006a).

A significant challenge in this area of research rests on methodological differences, where consensus regarding the definition of periventricular is lacking. A recent commentary on this issue points to an inherent problem with this definition, “Is the whole brain periventricular?” (Barkhof, F. & Scheltens, P., 2006) The commentary states that due to the confluent nature of pvSH, growth rates of these hyperintensities have a tendency to extend in a contiguous manner into the deep white regions, thus enveloping previously defined dwSH. Similar to van den Heuvel group’s report of pvSH making up 80% of total SH (van den Heuvel, D. M. et al., 2006a), results from this thesis show pvSH making up 90% in ADs, and 87% in NCs. In contrast to these reports, the
earlier Rotterdam study using a 10mm perimeter cutoff for pvSH report pvSH making up 60% of total SH (De Groot, J. C. et al., 2002).

In light of these methodological differences, it is recommended that consensus criteria be developed regarding a proper and anatomically meaningful definition to distinguish between these two sub-types. Given the potential differences in underlying vasculopathy in relation to location, it may be beneficial to consider definitions based on arteriolar and venular systems. As there are limited reports in the literature regarding progression of pvSH and dwSH and the various methodological differences in defining these lesions, it is clear that future research into this area is required to fully understand this long standing debate.

6.5 Whole Brain vs. Strategic: Does Location Matter?

Along the same lines of this debate, the question of whether to examine whole brain SH vs. strategic SH within different locations of the brain remains. The debate continues as evidenced by different approaches from 2 recent longitudinal studies, one limited to whole brain SH volumes irrespective of location (Carmichael, O. et al., 2010), and the other using a voxel based location approach (Smith, E. E. et al., 2011). In the voxel based approach, voxel clusters of SH in the temporal and parietal regions were associated with episodic memory deficits, while SH clusters in the inferior frontal regions were associated with executive dysfunction (Smith, E. E. et al., 2011). As further support for the SH location theory, an association with SH volumes in the right temporal lobe and performance on a numerical memory task using ROI-based regional delineations (Burton, E. J. et al., 2004). Results from this thesis support the SH location
theory, where SH volumes in particular regions were differentially associated with performance on different cognitive tasks.

Further support regarding the importance of SH location comes historically, dating back to Binswanger, with theories pointing to ischemia and small vessel disease (Blass, J. P., Hoyer, S., & Nitsch, R., 1991). While the brain’s vascular architecture has a large and rich blood supply for the gray matter, the white matter is generally supplied by longer and smaller arteries and arterioles, thus making it more susceptible to injury due to ischemia (Munoz, D. G., 2006; Wodarz, R., 1980). Regional differences in blood supply, such as the periventricular region, may make particular areas of the brain more susceptible to leukoariosis, and thus explain why SH are generally found in particular regions of the brain’s white matter. Future research directly exploring ischemia and SH is needed to further examine the relationship between leukoariosis and vasculopathy, including clinical, brain imaging, and pathological correlation.

Thus, the clinical significance of white matter vasculopathy remains somewhat controversial (Decarli, C. et al., 1995; Decarli, C. et al., 1996; Erkinjuntti, T. et al., 1994). Even the question of whether SH contribute independently to cognitive deficits over and above global atrophy remains unclear due to conflicting results (Esiri, M. M., Wilcock, G. K., & Morris, J. H., 1997; Looi, J. et al., 2002; Mungas, D. et al., 2001; Mungas, D., Reed, B. R., Ellis, W. G., & Jagust, W. J., 2001). This issue has begun to be addressed by quantifying both atrophy and SH burden simultaneously (Fein, G. et al., 2000; Laakso, M. P., 2002; Swartz, R. H., Stuss, D. T., Gao, F., & Black, S. E., 2008; Wiseman, R. M. et al., 2004). Similarly, the LE pipeline described in this thesis was designed to allow for an accurate atrophy measure of ST-TIC, which accounts for all
intracranial CSF superior to the tentorium (Levy-Cooperman, N., Ramirez, J., Lobaugh, N. J., & Black, S. E., 2008; Ramirez, J. et al., 2011; Ramirez, J., Gao, F. Q., & Black, S. E., 2007). Using the brain parenchymal fraction (BPF) as an indicator of global atrophy, this study confirms that atrophy contributes the largest proportion of the variance measured, estimated to account for 15-26% of the variance, followed by age and education, with SH volume accounting for less than 5% overall (See Table 4.2).

6.6 Lacunes: Consensus?

Recent controversies regarding the definition and identification of lacunes has renewed interest in this lesion sub-type, often referred to as silent brain infarcts (Potter, G. M. et al., 2010; Potter, G. M., Marlborough, F. J., & Wardlaw, J. M., 2011; Wardlaw, J. M., 2008). The Leukoariosis and Disability study (LADIS) reported that 47% of 633 elderly over the age of 65 had at least one lacune, with thalamic lacunes associated with poorer memory, speed and motor control, and executive function, independent of the WMH volume (Benisty et al., 2009).

Participants in the PATH Through Life community-based longitudinal study (n=477) showed a significant increase in lacunar infarct volume over the course of 4 yrs (Chen, X., Wen, W., Anstey, K. J., & Sachdev, P. S., 2009). Although lacunar infarction has been associated with a number of risk factors, hypertension appears to be a common finding (Chen, X., Wen, W., Anstey, K. J., & Sachdev, P. S., 2009; Das, R. R. et al., 2008; Prabhakaran, S. et al., 2008; Vermeer, S. E. et al., 2003a; Vermeer, S. E., Longstreth, W. T., Jr., & Koudstaal, P. J., 2007). Some studies suggest that SH may be more related to diffuse cognitive decline, while multiple silent lacunar infarcts may be more associated with frontal lobe dysfunction (Koga, H. et al., 2009). Unfortunately, as
described in a recent review, wide variability in definition and quantification of lacunes call for consensus criteria to be established (Potter, G. M., Marlborough, F. J., & Wardlaw, J. M., 2011).

In this thesis, lacunes were defined as cystic fluid-filled lacunar-like infarcts, defined by a set of voxels greater than 3mm in diameter that met a hyperintense criteria on both PD and T2 and a hypointense to CSF criteria on T1. These criteria were in harmony with previous reports in the large Cardiovascular Health Study (Longstreth, W. T., Jr. et al., 1998) and Rotterdam Study (Vermeer, S. E. et al., 2003b; Vermeer, S. E., Longstreth, W. T., Jr., & Koudstaal, P. J., 2007).

Due to the small volumes found in our population, the results contribute little to this debate, as no associations were found with lacunar volume and the neuropsychological tests used. While the extremely small volumes of lacunes (AD=0.4; NC=0.1), the small distribution, and numerous zero values, may have limited the correlative power in this thesis, it is possible that future associations may be found with the inclusion of additional neuropsychological tests and longitudinal data. Finally, it is recommended that future examinations of lacunes should adopt a similar procedure that was implemented in the LE pipeline, as it provides a less arbitrary approach that is less susceptible to errors.

6.7 Thesis Relevance

In a recent incidence report published by the Alzheimer Society of Canada, between 5-8% of Canadians over the age of 65 have Alzheimer’s Disease, rising to 30-50% of those over 85 (Diamond, J., 2009). As Canada’s largest population cohort, the “baby-boomers” (people born between 1946 and 1965), enters senior citizenship, the
overall incidence of Alzheimer’s Disease and related dementias increases significantly, posing a major strain on Canada’s health care system (Alzheimer Society of Canada, 2010). While various approaches, from genetics to art therapy, seek to address this impending problem, the studies outlined in this thesis provide insight to the potential utility of neuroimaging for quantifying the conjoint burden of AD atrophy and small vessel disease, both of which are ubiquitous in the aging population and must be better understood in order to make progress.

In this thesis, neuroimaging allowed us to examine the complex interplay between normal aging, leukoariosis, AD, and dementia. While primarily a volumetric series of analyses, relationships with lesion location and performance on different cognitive tasks were also demonstrated using a systematically validated MRI processing pipeline - Lesion Explorer. Additionally, the results from this thesis provide a framework for future research, allowing for the integration of other imaging techniques and approaches.

Recent innovations with diffusion imaging revealed that the observable hyperintensities on structural MRI are merely the tip of the iceberg. Advances in neuroimaging techniques provide an ever-changing window, increasing our resolution, as we seek to understand the aging human brain and neurological diseases. Mixed pathologies, such as AD and cerebrovascular disease, are an ever-present reality and add to the complexity when attempting to understand dementia. Non-invasive neuroimaging such as MRI, allow us to examine normal aging, AD and dementia in vivo, which will assist in the development of better clinical diagnostic tools, identify important
risk factors, and help guide clinico-therapeutics towards lessening the burden of this debilitating disease facing elders in Canada and around the world.

Reference List


Ref Type: Abstract


