SUBCORTICAL ISCHEMIC VASCULOPATHY IN ALZHEIMER'S DISEASE:
BRAIN- BEHAVIOUR RELATIONSHIPS

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy,
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ABSTRACT

Brain- Behaviour Relationships of Subcortical Ischemic Vasculopathy in Alzheimer’s Disease

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The presence of white matter hyperintensities (WMH) and silent infarcts on magnetic resonance imaging is a common finding in elderly individuals. This subcortical ischemic vasculopathy is associated with age and cerebrovascular risk factors and can increase the risk of dementia, yet the contribution of subcortical vascular disease to the clinical profile and progression of Alzheimer’s disease patients is still relatively poorly understood. This study assessed the presence, severity and progression of WMH and lacunar infarcts and studied their relationship with measures of brain function and cognition in 64 patients with Alzheimer’s disease. Both a visual rating scale and volumetric tissue segmentation analysis were used to evaluate brain-behaviour relationships of WMH seen on T2-weighted and Proton Density MRI scans. In addition to describing the topographical distribution of WMH and lacunes, and examining sex differences, the volume and location of WMH were correlated with executive function, frontal lobe perfusion, and medial temporal lobe atrophy. The results confirm and extend previous findings suggesting that WMH are located primarily in the frontal and parietal regions and are associated with mild decline in tasks of executive function. WMH severity was not associated with a decrease in frontal lobe perfusion as measured by Single Photon Emission Computed Tomography. The investigation of different WMH subtypes revealed that lacunar infarcts were found most often within deep WMH. At one year follow-up, progression was seen in deep WMH, specifically in the frontal lobe and in lacunes found within the periventricular regions. Furthermore, progression in WMH
was associated with a decline in cognition. Taken together, these studies indicate the utility of measuring WMH by subtype (periventricular and deep WMH and lacunes) in understanding progression patterns and brain-behavior relationships. Since, subcortical ischemic vasculopathy may be potentially preventable; this study underlines the need to study interventions that address risk factors for the development of small vessel cerebrovascular disease, which may be helpful in preventing disability in the elderly.
ACKNOWLEDGMENTS

I am extremely grateful to a number of individuals who have lent their support, guidance, advice and friendship throughout the process of researching, writing and defending this thesis.

The supervision and mentorship of Dr. Sandra Black has been instrumental in my growth as a scientist. Dr. Black’s vision is what lead me to pursue graduate training and prompted me to continuously challenge myself with new topics and fields of research, from neuropsychology to medical imaging. Her work as a clinician and a scientist is renowned and one only needs to see how she interacts with her patients to understand why she is an inspiration to so many budding young physicians and scientists. I am so thankful to have been exposed to her passion for science, medicine and teaching, and most importantly her compassion and thoughtfulness with her students, staff and patients. I am grateful for the knowledge, energy and most importantly the constant support, even through the struggles, that she gave me. I feel so lucky to have had the opportunity to develop both professionally and personally under her tutelage.

I would also like to thank my thesis committee for their insight, suggestions and challenging questions throughout every meeting. Dr. Curtis Caldwell helped me to develop my confidence when answering difficult questions, and also encouraged me to simplify my writing and my oral responses. A special thank you to Dr. Nancy Lobaugh who spent countless hours reviewing the intricacies of my data, methodology and statistical analyses with me, and who also provided critical feedback on my writing. I feel that I have grown into a stronger scientist and writer due to my committee’s support.

Several other individuals have given their time to provide assistance and feedback in the final stages of my training. First, I would like to thank my external reviewer Dr. Michael O’Sullivan who provided constructive criticism and input into the review of my thesis and also thoughtful and insightful questions during my oral defense. I enjoyed discussing white matter disease with Dr. O’Sullivan at both VASCOG meetings and am pleased that he was available to participate in my defense over the phone from England. I would also like to thank Dr. Bruce Pollock for his role as internal reviewer. His interesting questions prompted me to explore different dimensions of my data that improved the final product.

I cannot express my gratitude for the professional and personal support I received from my colleagues in the L.C. Campbell Cognitive Neurology Unit. Special thanks to Joel Ramirez without whom I would not have been able to complete countless hours of white matter hyperintensity measurement and analysis. I am so grateful for Joel’s support and enjoyed our scientific discussions and mock committee meetings. I truly could not have done this without his help and encouragement. Special thanks also to Farrell Leibovitch for his advice, friendship and patience with my statistics inquiries and to Dr. Fuqiang Gao for his assistance in lacune measurement and CHIPS ratings, and also for his willingness to assist and explain the complexities of brain vasculature. Thank you to Christopher Scott for assisting with brain volumetry and also for his scientific insight, advice and companionship in the heat of the imaging lab. Special thanks to my colleagues Vessela Stamenova, Dr. Neelesh Nadkarni, Dr. Mario Masellis, Dr. D.J. Sahlas, Dr. Jennifer Mandzia and Dr. Sarah Duff-Canning who have all provided thoughtful discussion and critical input to many important issues that arose as my research
progressed and who also offered friendship and comic relief during many stressful moments.

I am especially grateful to the research assistants, psychometrists and study coordinators of the Cognitive Neurology group, especially Isabel Lam, Isabelle Guimont and Joanne Lawrence. The administrative support of Loren Kannegiesser, Tatiana Brezden, Malcolm Morum and Libby Duke has been a great help. Words cannot express how thankful I am to have met and become close friends with Jennifer Bray. Jennifer has not only been my personal support system over the past seven years, but has offered professional advice, editing and assistance (no matter how trivial) with all aspects of my academic pursuits. She is a remarkable person and I am truly lucky to have such a wonderful friend and colleague.

The data acquisition for this project was funded primarily through operating grants from the CIHR, but also Ontario Mental Health Foundation, the Alzheimer’s Association-US and the Alzheimer’s Society-Canada, Personal support has been provided by the Alfred A. Aberman OGSST, the Scace Graduate Fellowship for Research in Alzheimer’s Disease, the Margaret and Howard Gamble Research Fellowship, the Paul and Adelle Deacon Graduate Scholarship in Science and Technology, the K.M. Hunter Graduate Scholarship and the L.C. Campbell Cognitive Neurology Research Unit. This work also benefited from infrastructure support from the Heart and Stroke Foundation Centre for Stroke Recovery.

Lastly, I could not have made it through my training without the support and commitment of my friends and family. I dedicate this thesis to my incredible parents who have stressed the quest for knowledge in all their children from a very young age. I appreciate their support, their friendship, their advice and their constant love as I know I could not have gotten through this process without them. I would also like to thank my siblings who were always available with words of encouragement or just to lend an ear and share in my joys and frustrations. I would like to thank my close network of friends, especially Dana Levitt and Jennifer Kicis who constantly grounded me during moments of stress. Finally, to my loving husband and partner Levi Cooperman, who is my greatest supporter and who never ceased to share his time or efforts in helping me get through this degree. I could not have succeeded without him by my side, helping on numerous fronts, from formatting to sitting through my practice talks. I am so grateful for his friendship, kindness, encouragement, patience and love.
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<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>ARWMC</td>
<td>Age-related white matter changes scale</td>
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<td>CADASIL</td>
<td>Cerebral Autosomal Dominant arteriopathy with subcortical infarcts and leukoencephalopathy</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CVD</td>
<td>Cerebrovascular Disease</td>
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<td>DRS</td>
<td>Dementia Rating Scale</td>
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<td>DWH</td>
<td>Deep white hyperintensities</td>
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<td>ECD</td>
<td>(99m) Tc-Ethyl cysteinate dimer</td>
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<td>EF</td>
<td>Executive Function</td>
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<tr>
<td>FAS</td>
<td>Phonemic fluency (for words beginning with F, A, and S)</td>
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<td>FDG-PET</td>
<td>18F-fluorodeoxyglucose positron emission tomography</td>
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<td>FLAIR</td>
<td>Fluid Attenuated Inversion Recovery</td>
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<td>GM</td>
<td>Grey matter</td>
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<td>HMPAO</td>
<td>Tc-hexamethylpropyleneamine</td>
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<td>ICC</td>
<td>Inter-class correlation</td>
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<tr>
<td>MANCOVA</td>
<td>Multiple analysis of covariance</td>
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<tr>
<td>MANOVA</td>
<td>Multiple analysis of variance</td>
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<td>MMSE</td>
<td>Mini-Mental Status Examination</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MTL</td>
<td>Medial temporal lobe</td>
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<td>NAA</td>
<td>N-Acetyl Aspartate</td>
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<td>NINDS-ADRDA</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer’s Disease and Related Disorders Association</td>
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<tr>
<td>PD</td>
<td>Proton density</td>
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<td>PVH</td>
<td>Periventricular hyperintensity</td>
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<td>rCBF</td>
<td>Regional cerebral blood flow</td>
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<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SABRE</td>
<td>Semi-automated brain region extraction</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SH</td>
<td>Subcortical hyperintensities</td>
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<td>SIVD</td>
<td>Subcortical Ischemic Vascular Disease</td>
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<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
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<td>TIA</td>
<td>Transient ischemic attack</td>
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<tr>
<td>TIV</td>
<td>Total intracranial volume</td>
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<tr>
<td>TRV</td>
<td>Total regional volume</td>
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<tr>
<td>VaD</td>
<td>Vascular Dementia</td>
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<td>WCST</td>
<td>Wisconsin Card Sorting Task</td>
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<td>WM</td>
<td>White Matter</td>
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<tr>
<td>WMH</td>
<td>White Matter Hyperintensities (includes periventricular, deep white and deep nuclei hyperintensities)</td>
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<td>YOE</td>
<td>Years of education</td>
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CHAPTER I
THESIS INTRODUCTION

White Matter Hyperintensities

The increasing availability of brain imaging studies over the past three decades has revealed the frequent and unexpected presence of diffuse lesions in the cerebral white matter of both asymptomatic and cognitively impaired elderly individuals (Kertesz et al., 1988; Fazekas, Chawluk, Alavi, Hurtig, & Zimmerman, 1987; DeCarli, 2003). These areas of increased signal intensity, so-called white matter hyperintensities (WMH) on Proton Density (PD), T2-weighted (T2) and Fluid Attenuated Inversion Recovery (FLAIR) magnetic resonance imaging (MRI), usually indicate the presence of small vessel ischemic vascular disease, and are particularly common in individuals with cerebrovascular risk factors or stroke (Pantoni & Garcia, 1995; Capizzano et al., 2004; Kalaria, 1999). However, the high prevalence of WMH observed in healthy individuals over fifty years of age have raised the question of whether white matter lesions may be a normal age-related phenomenon (DeCarli et al., 1995).

White matter has, for purposes of imaging studies, often been subdivided into the zone under the cortex (deep white matter) and the area surrounding the ventricles (periventricular white matter). The white matter immediately adjacent to the cortex has a high density of U-fibers, which connect neighboring cortical areas, while the periventricular region contains long association fibers that connect anterior and posterior cortices and cortical areas to subcortical nuclei (de Groot et al., 2000). The extent of these WMH may range from narrow rims or caps surrounding the ventricles, to patchy subcortical areas which can become large enough to encompass the entire centrum semiovale (Kertesz et al., 1988). The exact causes of white matter pathology have not
been conclusively established. Pathophysiological origins of WMH are diverse and include multiple cerebrovascular and neuropathological factors. Abnormal white matter signal hyperintensities have been associated with white matter rarefaction (Pantoni et al., 1995), dilation of perivascular spaces, gliosis (Brun & Englund, 1986), demyelination, clasmatodendrosis (cytoplasmic swelling and vacuolation of astrocytes) (Sahlas, Bilbao, Swartz, & Black, 2002), amyloid angiopathy, and small deep white matter microcystic and discrete infarcts (Smith, Snowdon, Wang, & Markesbery, 2000). Some research suggests a relationship between WMH and periventricular venous collagenosis, which suggests that some incidental hyperintensities are related to chronic brain edema from leaky vessels (caused by a build up of collagen within the periventricular veins) (Moody, Brown, Challa, & Anderson, 1995; Moody, Brown, Challa, Ghazi-Birry, & Rebourssin, 1997). Another possibility that has been reported involves cerebral amyloid angiopathy, or the accumulation of Amyloid-β in small vessels (Tomimoto et al., 1999; Pettersen et al., 2008). This can lead to smooth muscle loss, decreased vasoreactivity, rupture and can result in WMH in AD.

A landmark pathoanatomical study described WMH microscopically in a sample of eighty-four dementia patients (Brun et al., 1986). The authors found the “white matter disorder” to be associated with partial loss of axons, myelin sheaths, and oligodendroglial cells. White matter lesions were also ranked in three steps from normal to completely destroyed tissue with loss of oligodendroglial cells and axons, reactive astrocytosis, and arteriolar changes all increasing gradually in extent and severity but not reaching complete destruction. The topographic distribution was also described as predominantly symmetrical extending from the periventricular region in all directions.
toward the periphery. In severe cases, the entire white matter was included sparing only U fibers (Brun et al., 1986).

WMH have also been characterized according to their appearance and location in the brain on brain mapping. Most often they are reported either as smaller punctate lesions or early confluent to confluent lesions. The majority of confluent lesions are typically found in the arterial watershed areas of the centrum semiovale, while punctate lesions are more often found in a diffusely scattered pattern in the deep white matter (Enzinger et al., 2006). These hyperintensities are typically labeled as “deep white hyperintensities” (DWH) or “periventricular hyperintensities” (PVH) depending on the location in the brain that they occupy. It should be noted, however, that the nomenclature for hyperintensities, much like the research results, is quite controversial. For example, because WMH are found in both white matter and also subcortical regions such as the basal ganglia, they are also referred to by some authors as subcortical hyperintensities meaning all hyperintensities between the cortical rim and the ventricles (Levy-Cooperman, Lobaugh, Caldwell, Gao, & Black, 2007a; Mori, 2003) (Cohen et al., 2001). For consistency and to simplify terminology, the term WMH throughout this thesis will be used to include hyperintensities in the deep white matter and deep nuclei (basal ganglia and thalamus) and periventricular regions.

The clinical significance of WMH has been unclear as they have been associated with brain atrophy (Capizzano et al., 2004), focal neurological signs, gait disorder, and in some but not all studies, poorer neuropsychological test performance (Capizzano et al., 2004) (DeCarli et al., 1995). Thus far, the exact cause of hyperintensities not due to infarction remains elusive. With the introduction of advanced imaging techniques such
as diffusion tensor imaging, magnetic resonance spectroscopy and brain volumetry may begin to shed light on these complex finding.

**Measurement of WMH**

*Visual Rating Scales*

The measurement of WMH has been a source of controversy due to the variety of qualitative visual rating scales and quantitative imaging techniques being employed in order to assess the severity of this pathology. Earlier studies typically relied on visual rating scales to measure WMH as they were fast, efficient and were easily accessible. More recently visual rating scales have become especially popular for large-scale multi-site studies where sample sizes are large and different scanners are used within each study (Pantoni et al., 2005). Researchers have a number of visual rating scales to choose from, with some distinguishing between PVH and DWH and others combining the two. One of the most commonly used scales, created by Fazekas and colleagues, distinguishes between PVH and DWH, with PVH graded from 0 (absent) to 3 (irregular PVH extending into the deep white matter) and DWH rated as 0 (absent) to 3 (large confluent) (Fazekas et al., 1987). Scheltens designed a scale that also accounts separately for PVH and DWH, evaluating the presence and extent of PVH and DWH in different anatomic regions with a 0-6 point scale. The scale bases the score on the size in millimeters of the hyperintensities. In addition to MRI rating scales, separate scales specific to CT-scans are also available. Although visual rating scales are less time-consuming than quantitative measures, they are heterogeneous in regard to their range and exact morphological description, specifically, the diameter of lesion and the distinction between PVH and DWH (Pantoni et al., 2002). As a result, a consensus-derived rating scale developed under the auspices of the European Task Force in Age-Related White
Matter Changes (ARWMC) was introduced in order to address the heterogeneity of so many different scales (Wahlund et al., 2001). The ARWMC scale combines PVH and DWH and gives separate scores for five bilateral brain regions; however, it is important to note that this scale is still not used exclusively in large studies (Wahlund et al., 2001; Pantoni et al., 2002). It is possible that the variability in rating scales has contributed to some of the inconsistencies in the literature, especially with regard to the relationship between WMH, cognition, and function (Mantyla et al., 1997; Wardlaw, Ferguson, & Graham, 2004).

**WMH Volumetrics**

Quantitative measurement of WMH uses computer-based techniques to obtain volumetric measures of WMH burden (Quddus et al., 2004; Admiraal-Behloul et al., 2005; Wu et al., 2006; Wen & Sachdev, 2004). In manual tracing procedures, a trained rater reviews a scan on a computer console and manually traces WMH using the cursor. These volumes are then calculated based on the thickness and the number of pixels in the traced area and summed to get a total WMH intensity volume. This technique is sensitive but time consuming as each brain can take hours to complete. Both semi- and fully automated techniques are based on intensity-based computer algorithms for segmenting the WMH. These algorithms count the number of voxels that exceed either a predefined intensity threshold or a calculated intensity cut-off for putative hyperintensities (Admiraal-Behloul et al., 2005; DeCarli, Fletcher, Ramey, Harvey, & Jagust, 2005; Wu et al., 2006; Wen et al., 2004). The difference between automated and semi-automated occurs after the intensity algorithm is applied. Semi-automated techniques involve the manual selection of relevant hyperintensities (Quddus et al., 2004; Wen et al., 2004). Although this approach can also be time-consuming, it greatly reduces the risk of false-
positives reducing the risk of inflated WMH volumes that are often generated by fully-automated techniques.

Wen and Sachdev (2004) introduced a WMH quantitative technique that used FLAIR as the target and T1-images as a reference for the removal of false WMH detection; however, they also included further visual inspection of each WMH map. This study also included topographical information on WMH distribution. Each brain was normalized into Talairach space and a profile of WMH distribution by region-of-interest (ROI) was tabulated (Wen et al., 2004; Yoshita, Fletcher, & DeCarli, 2005). Another semi-automatic technique uses a K-Means cluster analysis in order to segment the entire brain into different tissue compartments (cerebrospinal fluid (CSF), white matter (WM), and grey matter (GM)). This process is then followed by manual editing on a slice by slice basis in which WMH which the K-means procedure segmented as either GM or CSF due to their relative hyperintensity manually reclassified (Fein et al., 2000; Swartz et al., 2002). DeCarli and colleagues also utilized a semi-automatic technique as part of an extensive algorithm to acquire morphometric data for a large population study (DeCarli et al., 2005). Images were segmented by performing slice-based inhomogeneity corrections and brain matter segmentation on T1 scans. It should be noted that manual intervention was used to remove non-brain tissue from the images (Yoshita et al., 2005). Automatic WMH segmentation techniques with no human intervention have been little used until recently when one group reported a fully-automated segmentation technique. The technique requires information from three different MR-images (PD, T2 and FLAIR); however, it replaces human validation with an established artificial intelligent technique (fuzzy-logic algorithms) (Admiraal-Behloul et al., 2005). In this 2-level
algorithm, intensity values were assigned linguistic criteria (“dark” or “bright”) based on probabilistic estimates from grey and white matter templates. The final step involved a reasoning level in which the linguistic criteria were used to deduce the appropriate tissue class. Although this technique seems promising, its accuracy and rate of error has not fully been evaluated.

Although many studies have employed methods of quantification to determine severity of WMH, very few have provided information on the location or type of lesion (lacune, confluent, periventricular rims or caps). Repeatedly, the above mentioned techniques combine hyperintensities seen in the basal ganglia with those of the frontal lobes without differentiating between lacunes and WMH. Although one study suggested that regardless of location, WMH are associated with hypometabolism in the frontal lobe, the regions examined were large and differences between PVH and DWH were not examined (Tullberg et al., 2004). Thus, further study on the effect of WMH in separate brain regions is needed. Some studies report that mainly PVH, rather than DWH are associated with cognitive impairment (de Groot et al., 2000). Yet in an autopsy study conducted on a sample of elderly nuns, PVH were not significantly related to white matter volume, or dementia (Smith et al., 2000). Specific quantitative techniques that can distinguish between the different types of WMH would be needed to better understand brain-behavior correlations of WMH.

Measuring the Progression of WMH

With many large ongoing population studies in North America and Europe heading into the five year range, the amount of literature examining the progression of WMH is likely to increase dramatically. It is currently thought that WMH progress
gradually over time, with limited studies reporting varied results (Schmidt, Schmidt, Kapeller, & Fazekas, 2003b; Prins et al., 2004). Progression has been measured using visual rating scales, with some studies using scales specifically designed to measure progression (Prins et al., 2004), while others used a change in score to indicate degree of progression (de Leeuw, Barkhof, & Scheltens, 2005; Schmidt et al., 2003b). A review of visual rating scales, however, suggested that they were not well suited for measuring change since they did not correlate with volumetric measures and had low interobserver agreement (Prins et al., 2004). Nevertheless, these scales will likely continue to be used for the measurement of longitudinal change as many large site studies are unable to conduct volumetric analyses on images taken from multiple scanners due to scanner variability. To date, only a small handful of studies have measured progression using volumetric measures, with differing results. Most studies have simply subtracted volumes to examine whether there was any change in volume (Burton, McKeith, Burn, Firbank, & O'Brien, 2006) after accounting for head size. One study conducted a voxel-by-voxel comparison at time 1 and 2 using statistical parametric mapping (Sachdev, Wen, Chen, & Brodaty, 2007). In general, volumetric measurement of WMH progression is time-consuming and costly but, may be the most reliable measure since visually estimating change may be inaccurate due to human error. Although current data varies, research suggests that WMH may progress with time, but not in every individual and not in every location (Prins et al., 2004; Sachdev et al., 2007). Some studies have shown increases only in confluent WMH (Schmidt, Enzinger, Ropele, Schmidt, & Fazekas, 2003a), while others suggest that DWH progress at a faster rate (Sachdev et al., 2007). However, the length of follow-up has varied between studies, making
comparisons difficult. One consistent finding amongst both visually measured and volumetric studies is that the greatest risk for progression of WMH is the presence of baseline WMH (de Leeuw et al., 2005; Sachdev et al., 2007; Schmidt et al., 2003a; Schmidt et al., 2003b; van den Heuvel et al., 2004). Baseline presence of WMH could therefore be a legitimate treatment target in patients with cognitive complaints; including those with Alzheimer’s Disease (AD) patients.

**WMH in Normal Aging**

WMH of varying extent and distribution are commonly seen in the brains of clinically healthy elderly individuals. Population studies report a prevalence of MRI-detected WMH ranging from 39-96% (Soderlund, Nyberg, Adolfsson, Nilsson, & Launer, 2003; de Groot et al., 2000) in individuals over 60 years, and though hyperintensities are usually less severe than in demented patients, research suggests that they are related to cerebrovascular risk factors, specifically hypertension (Pantoni et al., 1995). Soderlund et al. (2003) suggest that the development of white matter damage is a slow process, enhanced by long standing elevated blood pressure. Hypertension plays a role in the early changes affecting the long penetrating arteries of the white matter (Ostrow & Miller, 1993). Arteriosclerotic changes begin to affect many cerebral blood vessels (lumen of small penetrating arterioles are narrowed due to the build up of hyaline material), leading to decreases in blood flow and possibly ischemic injury to the periventricular white matter (Pantoni et al., 1995; Libon, Price, Davis, & Giovannetti, 2004). As individuals age, blood pressure increases and may contribute to increased WMH (Pantoni & Garcia, 1997). This etiology does not cover all instances, however, because WMH are also present in some people independent of the presence of vascular risk factors (Pantoni et al., 1995; Sachdev, Wen, Christensen, & Jorm, 2005). There may
be a genetic loading for WMH on chromosome 4 (DeStefano et al., 2006; Atwood et al., 2004) and there is an autosomal dominant disorder Cerebral Autosomal Dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) in which a gene defect in the cerebral arteries leads to fatal vascular disease (Tournier-Lasserve et al., 1993; O'Sullivan et al., 2001; Chui, 2007).

DeCarli et al. (1995) examined the significance of WMH in a sample of 51 healthy adults aged 19-91 years without cerebrovascular risk factors. This study investigated WMH effects on cognition, general health and also specific regional glucose metabolism using positron emission tomography using 18F-fluorodeoxyglucose (FDG-PET). WMH volume was a significant independent predictor of general intelligence, visual memory task scores, and tests of frontal lobe function (Trails A and B). However, WMH were not a significant predictor of regional cerebral glucose metabolism in any of the brain regions examined. The study also aimed at examining how volume and severity of hyperintensities influenced various measures of both brain and cognitive function. Previous research had suggested that a threshold level of WMH severity on MRI may be needed before WMH have an effect of cognitive or brain function (Boone et al., 1992; Herholz et al., 1990). Upon further investigation of quantitative data, large WMH volumes (greater than 0.5% of intracranial volume) were present in 10% of the study population but only in subjects greater than 50 years of age. Results showed that WMH continued to be a significant predictor of general intelligence, visual memory tasks, and tests of frontal lobe function even when they excluded patients with large WMH. This raised questions about the threshold effect theory. However, they also compared those patients with large WMH to an age matched sample with WMH volumes less 0.5% of
total intracranial volume. Those subjects with large WMH had significantly higher systolic blood pressure, lower cognitive scores specifically on frontal lobe measures, and lower frontal lobe metabolism (DeCarli et al., 1995). These findings should, however, be interpreted cautiously due to the small number of participants in the large WMH sample. Nevertheless, reduced frontal lobe metabolism and impaired frontal neuropsychological performance may indicate preferential impairment of frontal lobe neural circuitry in individuals with WMH, an inference supported by a more recent PET study showing that WMH, irrespective of location, correlated with frontal glucose metabolism (Tullberg et al., 2004).

The difficulty of distinguishing whether WMH represent a pattern of normal aging or some variant of cerebrovascular disease has prompted researchers in this field to explore new techniques to examine the data. Constans et al. (1995) described the metabolite alterations that are associated with hyperintense regions on MRI. Their study utilized Magnetic Resonance Spectroscopy to characterize WMH in the brains of elderly non-demented subjects. Their data suggested that hyperintense regions of the white matter were associated with increased signal intensity of choline peak with no apparent decrease in the N-acetyl aspartate signal, a marker of neuronal health. A trend toward lower percent phosphomonoester in the WMH was also reported suggesting that WMH may alter brain myelin phospholipids, with no significant loss of neuronal or axonal density. The lack of a lactate peak suggested an absence of ongoing brain ischemia. Interestingly, Constans et al. (1995) also investigated normal appearing white matter on the contralateral side of those patients with extensive WMH and found similar patterns although relatively less marked, suggesting that the process underlying WMH may be
more diffuse than revealed by MRI. In fact, WMH may represent a long standing process that takes years before it is evident on MRI and even longer before any cognitive deficits occur.

*Subcortical Ischemic Vascular Disease*

The term Subcortical Ischemic Vascular Disease (SIVD) is used to refer to the clinical presentation in which subcortical infarcts and white matter changes, but not large cortical infarcts are present (Reed et al., 2007; Erkinjuntti, 2003). The primary vascular mechanism in SIVD is thought to be small vessel disease (Erkinjuntti, 2003). SIVD is a subtype of Vascular Dementia (VaD) which implicates small vessel disease as the primary etiology and lacunar infarcts or white matter lesions in the subcortical areas as the main brain pathologies (Yoshikawa et al., 2003). SIVD is caused by small vessel change such as arteriolosclerosis, lipohyalinosis, and intracranial atherosclerosis. Two separate pathways can cause injury from small vessel disease; the first involves occlusion of a single arterial lumen leading to complete lacunar infarction, while the second involves stenoses of multiple vessels leading to hypoperfusion and incomplete infarction (Kalaria, 2002). These pathways translate into two typical neuroimaging findings: 1) WMH in subcortical locations, including PVH and DWH, and 2) lacunes or small infarcts with increased signal relative to CSF on PD-MRI that are surrounded by white matter or cortical grey matter, occurring in the caudate, globus pallidus, thalamus, internal capsule and frontal white matter (Erkinjuntti, 2003; van Straaten, Scheltens, & Barkhof, 2004). SIVD is now recognized as a common cause of cognitive impairment and possibly dementia in later life. It can be associated with a distinct pattern of decline in cognitive function, characterized by attention and executive impairments, though
verbal and non-verbal memory can also be affected (Reed et al., 2007), and some behavioral symptoms such as depression (O'Brien, 2006).

An important issue confronting differential diagnosis in dementia and a major source of heterogeneity is the frequent coexistence of AD and SIVD (Scheltens et al., 1992) or other neurodegenerative diseases and cerebrovascular disease (O'Brien, 2006; Roman, 2004). SIVD is a coincident finding in more than one third of patients with AD. Lacunar infarcts were associated with significantly greater likelihood (20X) of dementia in elderly nuns with concomitant pathology (Snowdon et al., 1997). Furthermore, increasing epidemiological evidence that common risk factors for stroke are also risk factors for AD makes the study of SIVD co-morbidity a high priority for AD research because it introduces the possibility of treatment options for AD that were previously limited to patients with cerebrovascular disease. The prevalence of “mixed” AD and cerebrovascular disease is more frequent than initially thought. In fact, trying to distinguish between neurodegenerative and cerebrovascular disease may not be clinically valid or useful since overlap is so common especially in the elderly (van der Flier, Barkhof, & Scheltens, 2007). In a large population autopsy series, 36% of the cases examined met pathological criteria for AD only, whereas 45% had additional neuropathological evidence for vascular lesions (Lim et al., 1999). Thus, while AD or SIVD alone are each risk factors for and direct causes of dementia, these highly prevalent pathologies, together, represent a potent danger to mental health and independence in older age (DeCarli, 2003; van der Flier et al., 2007; Jellinger & Attems, 2007).
WMH and the Frontal Lobe

Functional Imaging and WMH

As is true for all organs, energy metabolism in the brain is necessary for the basic processes of cellular activity. The working brain requires a continuous supply of glucose and oxygen to generate the electrical activity required for neuronal signaling. The coupling between neural activity and energy metabolism is the foundation of functional neuroimaging (Frackowiak, Friston, Frith, Dolan, & Mazziotta, 1997), which has made several advances in the last three decades. Functional brain imaging techniques such as PET and Single Photon Emission Computed Tomography (SPECT) are currently used for examining deficits in regional cerebral blood flow and metabolism in several neurologic diseases, and have been used to investigate the effect of WMH on frontal function.

The principles for PET and SPECT studies are similar; in that in both imaging techniques, a radionuclide is synthetically introduced into a molecule of potential biological relevance and administered intravenously to a patient. Subsequent brain uptake of the radiotracer is measured over time and used to obtain information about the physiological process of interest. The most common radiotracers used in brain PET studies are F18-2-fluoro-2-deoxyglucose, which measures FDG metabolism, and O–15 H2O-PET (Cummings, 1993), which is used in activation studies to measure cerebral blood flow as a correlate of neuronal activity (Albani et al., 2001). The radiotracers most often used in SPECT are (99m) Tc-Ethyl cysteinate dimer (ECD) or (99m) Tc-hexamethylpropyleneamine (HMPAO), which are used for measurement of brain perfusion by providing a relative flow distribution when referenced to the cerebellum (Camargo, 2001). PET has higher spatial resolution (typically 5–7 mm with PET and 10–
14 mm with SPECT), but is very expensive and less widely available, though this may be changing, while SPECT is less expensive and more widely available in most hospitals.

One study looked specifically at the relationship of WMH to cerebral blood flow using SPECT imaging (Ott et al., 1997). Forty AD patients underwent both SPECT and MRI scanning. WMH were rated using a visual rating scale which distinguished between periventricular and subcortical or deep white matter changes. No significant relationship between global SPECT perfusion and severity of WMH was found. A semi-quantitative analysis was also conducted in which 7 bilateral ROIs were created from areas of association cortex and cerebellum. No significant relationships were reported, but, patients with larger amounts of WMH tended to have higher perfusion in the frontal cortex (Ott et al., 1997). Like a recent PET study (Tullberg et al., 2004), it was suggested that patterns of relative cerebral blood flow seen on SPECT may be independent of disconnection effects caused by subcortical pathology.

To examine the effect of WMH in different brain regions, Tullberg et al. (2004) conducted a PET study in a sample of 78 subjects with lacunar infarcts and WMH. Volumetric measures of WMH in five large brain regions (orbitofrontal, prefrontal, dorsolateral frontal, parietal and occipitotemporal) were derived using tissue segmentation. WMH volumes were then correlated to regional glucose metabolism, but unlike the more general relationships investigated in previous studies, the authors predicted that WMH within a specific brain region would have stronger effects on glucose metabolism in the same region than on glucose metabolism in different regions. They found that WMH were far more prominent in the prefrontal region compared to the
other four brain regions examined, suggesting that prefrontal white matter may be particularly vulnerable to small-vessel disease. Frontal WMH are often located in the periventricular arterial border zones which are also highly susceptible to hypoperfusion (Suter et al., 2002). While greatest volumes of WMH were seen in the frontal lobes, larger volumes of both frontal and parietal WMH were associated with decreased frontal glucose metabolism suggesting that regardless of regional distribution, WMH exerted the greatest metabolic effects on the frontal lobes. This pattern was more prominent in non-demented patients, and not described in patients with dementia, suggesting that in these patients some of who may have had coexisting AD, the primary pathology (AD or subcortical infarcts) may exert effects on cortical function that outweigh the effects of the WMH, as suggested by Fein et al (2000) and Swartz et al (2007).

According to the Tullberg study, WMH may have little effect on individuals with a concomitant disease; however, the subjects in their study had only mild to moderate WMH. Based on previous data that suggested a threshold effect of WMH (Boone et al., 1992; DeCarli et al., 1995), DeCarli and colleagues (1996) investigated the effects of WMH in AD by comparing AD patients with and without WMH, specifically comparing patients with the most extreme differences in severity of WMH. They included AD patients with no/minimal WMH, patients with severe WMH, and a group of healthy elderly. This study also used PET imaging to examine differences in cerebral glucose metabolism and a visual rating scale to rate severity of WMH (Fazekas et al., 1987). Results showed that compared with healthy controls, both AD groups had lower cerebral metabolism; however, the two groups showed different patterns of regional cerebral metabolic rate for glucose. AD patients without WMH showed uptake patterns typical
for AD (parietal-temporal reductions), while patients with severe WMH showed a different pattern (higher frontal, parietal, and temporal metabolic ratios) than generally seen in AD patients. Unlike Tullburg’s study, this study showed that AD patients with WMH exhibit a different cerebral metabolic pattern than that typically seen in AD suggesting that the presence of WMH may indicate a second pathological process that could contribute to cerebral dysfunction.

Yang et al. (2002) conducted a SPECT study that examined regional cerebral blood flow (rCBF) differences between patients with SIVD and normal elderly controls. This study employed a semi-quantitative visual rating scale (Mantyla et al., 1997) to classify severity of WMH in the periventricular region and deep white matter. Decreased blood flow was reported in the right thalamus, left subcallosal areas, cingulate gyrus, bilateral superior temporal gyrus and left caudate in those patients with SIVD. The regions of decreased perfusion seen in this sample correspond to the areas involved in the prefrontal subcortical circuit, specifically the caudate and thalamus, suggesting that one of the pathological processes (WMH or lacunes) occurring in SIVD may be disrupting rCBF in pathways that are indirectly associated with frontal function.

Some research suggests that it is strictly subcortical cerebral infarcts that lead to cortical hypometabolism and hypoperfusion in frontal regions, especially when lacunes are located in the basal ganglia and deep white matter (Kwan et al., 1999). Reed et al. (2004) investigated the effects of WMH on cortical function using PET imaging and reported a relationship between lacunes and prefrontal hypometabolism, whereas WMH were related to global hypometabolism. Interestingly, in this study both lacunes and WMH were correlated with a decline in executive function (Reed et al., 2004).
Kwan et al. conducted a similar PET-FDG study that included group comparisons of healthy controls and three groups with subcortical lacunes (1999). Like previous studies, frontal deficits were reported, but subjects with lacunes showed hypometabolism in the right frontal region only. In addition, patients with dementia had significantly lower global glucose metabolism. This study was among the first to suggest a direct relationship between SIVD and frontal function in dementia; however, it did not address the issue of location of WMH. Nor was any mention made of what brain areas were predominantly affected by WMH or lacunes, other than that infarcts were in cognitively strategic locations (thalamus and caudate).

In summary, regardless of the type of imaging employed to investigate brain function in patients and normal elderly controls with WMH, the data almost always indicates preferential impairment of frontal lobe neural circuitry (DeCarli et al., 1995). Although, most studies suggest a decrease in frontal function, a few studies have reported that WMH do not correlate with frontal metabolism or blood flow and one study reported hyperperfusion in the frontal lobes of patients with WMH (Ott et al., 1997). An increase in frontal perfusion may be the result of a compensatory mechanism in patients with severe WMH. If individuals with WMH can activate additional neural resources in the prefrontal cortex, which has been seen in AD patients (Grady et al., 2003), then one might expect this pattern to emerge only in those patients with more severe WMH.

**WMH and Executive Function**

The disruption of frontal lobe function confirms a relatively large body of research implicating decreased executive dysfunction as the most common behavioral correlate of WMH. Attention and executive control functions, but not memory or verbal
abilities, were highly correlated with both decreased metabolism in frontal regions and N-Acetyl Aspartate (NAA) concentrations in the frontal white matter in one such study (Pugh & Lipsitz, 2002). WMH may particularly compromise executive functions (e.g. attention, planning, concept formation, mental flexibility) through involvement of frontal lobe regions (Gunning-Dixon & Raz, 2000), thereby accelerating cognitive decline with aging (Mann, Mohr, Gearing, & Chase, 1992). One mechanism by which subcortical damage has been associated with a decline in executive function is based on the highly integrated anatomical and functional circuitry between subcortical structures and the frontal lobes (Cohen et al., 2001). Some authors have suggested that the prefrontal cortex may be preferentially affected by the disease process. Others have postulated a functional disconnection between the prefrontal cortex and more posterior cortical areas (Pugh et al., 2002), as well as frontal-subcortical circuits, which mediate motor activity and behavior in humans (Cummings, 1998). Five parallel frontal-subcortical circuits that link specific regions of the frontal cortex to the striatum, globus pallidus and thalamus have been described (Cummings, 1998; Tekin & Cummings, 2002; Cummings, 1993). The frontal cortical regions from which the circuits originate include 1) the supplementary motor areas mediating somatomotor activity, 2) the frontal eye fields mediating extraocular movements and 3) three circuits that originate in prefrontal regions (a. dorsolateral prefrontal b. orbital frontal c. anterior cingulate). The three prefrontal sites (a, b, c) are associated with neurobehavioral syndromes. The dorsolateral prefrontal circuit is dedicated to executive function. The orbitofrontal circuit is involved in social behavior, and dysfunction in this circuit can produce disinhibition and tactless impulsive behavior. The anterior cingulate circuit maintains motivational states in
humans; therefore disorder of this circuit can be associated with apathy (Tekin et al., 2002). Pugh and Lipsitz (2002) suggest that small vessel vascular lesions may be the most significant cause for frontal-subcortical dysfunction among the elderly. Damage caused by these lesions is thought to disrupt the frontal subcortical circuits at either the basal ganglia or frontal white matter sites leading to the cognitive deficits often seen in patients with WMH (Gunning-Dixon et al., 2000; Pugh et al., 2002). Sachdev et al. (2002) conducted a Magnetic Resonance Spectroscopy study in which imaging and neuropsychological data were collected on a sample of 160 stroke patients and normal community volunteers with WMH present on imaging. They found specifically that frontal WMH were correlated with decreased performance on tasks of executive function and reduced levels of NAA, suggesting that WMH are not a dormant condition, but may cause cognitive dysfunction in older individuals by disrupting brain connectivity (Sachdev et al., 2000). It is possible that the finding that WMH correlate with executive dysfunction may be more accentuated in pure cases of VaD, while those that have concomitant AD present with a mixture of deficits including more memory complaints. A recent autopsy study questioned the relationship of executive function deficits and SIVD. The authors suggested that patients with autopsy-confirmed cerebrovascular disease were equally impaired in executive function and memory tasks, while patients with mixed AD and cerebrovascular disease exhibited predominantly memory impairment similar to patients with only AD pathology. Clearly, additional research is needed in order to determine the relationship between WMH and cognitive impairment.

**Thesis Outline and Hypotheses**

In light of the increasing recognition of the synergy between AD and SIVD, it is important to determine if the comorbidity as measured by WMH on MR imaging can
explain some of the heterogeneity in cognitive presentation and temporal trajectory in AD patients (Holmes, Cairns, Lantos, & Mann, 1999). The use of both volumetric and functional brain imaging techniques may help to understand the complex effects of location, size and severity of vascular and AD pathologies in human aging and dementia. This project seeks to understand the contribution of WMH to the clinical profile and progression of AD patients. To obtain this goal, we assessed the presence, severity and progression of WMH and lacunar infarcts and studied their relationship with brain function and cognition in patients with AD.

The current investigation was conducted within the Sunnybrook Dementia Study, a longitudinal observational neuroimaging study of AD and other dementias. Detailed demographic, medical history, neuroimaging and neuropsychological data from individuals with dementia, aged 60 and above, without co-occurring neurological or psychiatric diseases are included in these analyses. All participants underwent a brain MRI between 1998 and 2003, 31 of whom underwent a second MRI one year later. The present study utilizes both a visual rating scale and volumetric tissue segmentation analysis to evaluate potential effects of WMH seen on T2- and PD-weighted MRI scans on patients with AD. In addition to comparisons based on severity of WMH, the volume and location of WMH were used to predict executive function, frontal lobe perfusion, and medial temporal lobe atrophy. Until recently, WMH were investigated primarily using visual rating scales which are efficient and reliable methods with which to determine the extent of WMH in large groups. In chapter two, the severity of WMH was evaluated using a visual rating scale developed under the auspices of the European Task Force on Age-Related White Matter Changes. This scale was used to classify patients
into three groups according to the severity of their WMH. Relationships between severity of WMH and frontal lobe function using SPECT imaging and executive tasks were investigated. Two hypotheses were tested: 1) patients with SIVD and AD would show greater prefrontal perfusion deficits on SPECT imaging than AD patients without concomitant SIVD and 2) patients with SIVD and AD would show decreased performance on neuropsychological tests of executive function.

In Chapter Three, computerized quantification of WMH volumes is applied to the same carefully selected sample of AD patients who did not have any history of stroke or transient ischemic attack. Detailed clinical histories of cerebrovascular risk factors and additional neuropsychological test scores are also collected. Using a novel in-house semi-automated technique to quantify WMH, which was able to distinguish between deep white and periventricular hyperintensities in addition to lacunar infarcts, we generated descriptive data on the overall topography of WMH in an AD sample. Summaries of the descriptive, imaging and neuropsychological data collected and used for subsequent analyses are presented. The hypotheses for this chapter involve WMH regional distribution and their clinical correlates. First, it was hypothesized that cerebrovascular risk factors and female sex will be related to larger overall WMH volumes. The second hypothesis is that measures of brain atrophy will be positively correlated with volumetric measures of WMH. Relationships between measures of medial temporal lobe atrophy and WMH volume have previously been reported (van der Flier et al., 2004; de Leeuw, Barkhof, & Scheltens, 2004b; Du et al., 2005); however, other studies have not examined the relationship between medial temporal lobe atrophy and subtypes of WMH. The current study investigates the possible relationship between
WMH and measures of atrophy by examining PVH and DWH separately. Third, it is hypothesized that the types and locations of WMH will correlate with specific cognitive tasks associated with certain brain region, specifically, lacunes and DWH in frontal regions will be associated with decline in executive tasks, and large PVH will be associated with general cognitive decline.

As discussed above, a major need attracting new interest in SIVD research is the measurement of WMH progression. Very little is understood about the rate and patterns of WMH progression, or even if they progress at all. The fourth chapter uses the same semi-automated volumetric techniques to measure WMH in the sample of AD patients at one year follow-up. The focus is on volumetric increases in specific brain regions and on subtype of WMH. The few previous studies that have examined progression in WMH have focused on follow-up at three or six years and the results have suggested a mild to moderate progression in WMH volume (Schmidt et al., 2003a; Sachdev et al., 2007; Burton et al., 2006). It should be noted however, that these studies did not focus on progression of WMH in specific regions nor did they specifically examine AD patients. Therefore, it was hypothesized that at approximately one year follow up, little to no change will be seen in periventricular, deep WMH or lacunar infarcts in this AD sample. Lacunes have been previously associated with subtle cognitive dysfunction and decreased frontal lobe metabolism, more so than WMH (Reed, Eberling, Mungas, Weiner, & Jagust, 2001; Reed et al., 2004), but no longitudinal studies have investigated lacunar infarct progression using volumetric methods. As a result, the second hypothesis was that while small increases in volumes of WMH would not correlate with cognitive decline; increased lacunar volume would do so.
A brief summary and discussion of the major findings of the thesis project are included in the final chapter.

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Ref Type: Abstract


CHAPTER II*
WHITE MATTER HYPERINTENSITIES IN ALZHEIMER’S DISEASE: NO CLEAR RELATIONSHIP WITH EXECUTIVE FUNCTION AND FRONTAL PERFUSION ON SPECT

INTRODUCTION

Alzheimer’s disease (AD) is the most common neurodegenerative pathology in the elderly. While the underlying causes that lead to dementia in AD are not completely understood, the pathology is well-defined by the presence of amyloid plaques, neurofibrillary tangles (Braak & Braak, 1995) and by brain atrophy beginning in the medial temporal lobes and extending over time to isocortical regions (Geula, 1998). There is considerable heterogeneity in both the cognitive and clinical presentation of AD. Evidence is emerging to suggest that one source of this heterogeneity may be the frequent coexistence of AD and Cerebrovascular Disease (CVD) pathologies. In one community-based autopsy series (N=134), only 36% of the cases met pathological criteria for AD alone, whereas 45% had additional neuropathological finding of vascular lesions (Lim et al., 1999). In another community neuropathology study (N=209), 78% of the individuals whose average age at death was 85 years, had evidence of vascular lesions, irrespective of a history of dementia (Anonymous, 2001).

A common finding among individuals with CVD and co-existing AD is the presence of subcortical white matter hyperintensities (SH) on T2-weighted magnetic resonance imaging (MRI). SH are particularly common in individuals with cerebrovascular risk factors or stroke (Capizzano et al., 2004), but they also occur in healthy individuals over fifty years old and are increasingly prevalent with aging, suggesting they are an age-related phenomenon (DeCarli et al., 1995). The

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pathophysiological origins of SH are diverse and include multiple cerebrovascular and neuropathological factors, ranging from so-called “incomplete” infarction (Pantoni et al., 1995), dilation of perivascular spaces, gliosis, demyelination, clasmatoendodrosis (cytoplasmic swelling and vacuolation of astrocytes) (Sahlas et al., 2002), and small deep white matter microcystic and lacunar infarcts (Pantoni et al., 1995). SH are now thought to be part of a continuum of vascular-related brain injury, which, along with lacunar infarcts, is referred to as Subcortical Ischemic Vascular Disease (SIVD) (Kalaria, 2002).

SIVD is associated with prominent frontal lobe dysfunction, particularly compromising executive functions (EF) (e.g. attention, planning, concept formation, mental flexibility) (Gunning-Dixon et al., 2000). These deficits are thought to result from disruptions of the highly integrated anatomical and functional connections between subcortical structures and frontal cortices, specifically the dorsolateral prefrontal and anterior cingulate cortex (Gunning-Dixon et al., 2000). Some research suggests that SH may also functionally disconnect prefrontal cortex and more posterior cortical areas (Pugh et al., 2002). Executive dysfunction is also frequently reported in AD patients, whose cognitive profile ranges from memory impairment in single cognitive domains to global cognitive decline (Buckner, 2004). Few studies have attempted to determine if SIVD comorbidity, as measured by SH, contributes to the heterogeneous clinical presentations of AD, and the results of those studies have been contradictory (DeCarli et al., 1996; Tullberg et al., 2004; Amar, Bucks, Lewis, Scott, & Wilcock, 1996).

AD is associated with a distinct pattern of regional brain atrophy, as well as reduced blood flow and glucose metabolism, usually involving bilateral parietotemporal

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areas prior to frontal areas (Van Heertum & Tikofsky, 2003). The degree to which SIVD exacerbates these findings is not yet known. Imaging techniques such as Positron Emission Tomography (PET) and single photon emission computed tomography (SPECT) have been used to examine the complex interactive effects of SH location, size and volume on brain function in aging and dementia (Reed et al., 2004). One PET study examined AD patients with extensive periventricular SH and showed decreased regional blood flow in the frontal lobes and lower scores on EF tasks, relative to patients without extensive periventricular SH (DeCarli et al., 1996). On the other hand, another study reported no significant differences in regional cerebral blood flow (rCBF) in AD patients with SH (Ott et al., 1997). A possible explanation for the discrepant findings in these studies is that the definition and measures of SH differed. Some research suggests that total volume of SH may be the most important factor in determining whether a patient exhibits any cognitive or functional deficits (DeCarli et al., 1995; DeCarli et al., 1996). In one study, normal elderly subjects with SH greater than 0.5% of intracranial volume showed significantly lower frontal lobe metabolism on PET (DeCarli et al., 1995). Findings such as this suggest that a threshold amount of SH must be present to have an effect on cerebral function.

The aim of the current, cross-sectional study was to examine relationships between SIVD burden and a) SPECT perfusion in the frontal regions and b) executive functioning in patients with AD (N=63). Patients with probable and possible AD were divided according to SH severity into severe SH, moderate SH, and no SH groups. The hypothesis was that the severity of SH, measured on T2- and Proton-Density-weighted MRI, would correlate negatively with frontal perfusion ratios on SPECT analysis and

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with performance on executive function tasks. Two additional measures were also investigated: a) SH involving cholinergic projections which are thought to be associated with selective cognitive deficits in cognitively impaired patients (Swartz, Sahlas, & Black, 2003) and b) the presence of lacunar infarcts. Both these measures were also hypothesized to be related to decreased rCBF in frontal regions and decline in executive function.

METHODS

Participants

Subjects were part of the Sunnybrook Dementia Study and recruited from the Cognitive Neurology clinic at Sunnybrook Health Sciences Centre, a University of Toronto academic healthcare institution. All patients were fluent in English and had adequate visual and auditory acuity to complete neuropsychological testing. Patients in this study had historical profiles typical of AD, with insidious onset and gradual decline. The patients were enrolled in a longitudinal observational study using the same standardized protocol. All patients received a comprehensive clinical evaluation, including detailed medical history, neurological examination, routine laboratory investigation and neuropsychological testing with a standardized test battery. The presence of cerebrovascular risk factors was ascertained including: arterial hypertension, diabetes, hyperlipidemia, and “other 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 et al., 1984), and the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (American Psychologioical Association, 1994) criteria for dementia. The “Possible AD” diagnosis was based on the presence of

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sufficient subcortical cerebrovascular disease to contribute to dementia. It is important to note that no patient in this study met the criteria for Vascular Dementia (VaD). In fact, patients were excluded if they met the National Institute for Neurological Disorders and Strokes (NINDS) - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) criteria for possible or probable VaD, which requires focal neurological signs in addition to imaging evidence of CVD. Presence of “silent” cortical strokes on MRI also excluded the patient. A final inclusion criteria was that participants’ SPECT, MRI and neuropsychological testing was completed within a consecutive 3 month period.

**Neurobehavioral Assessment**

Cognitive function was assessed with a battery of neuropsychological tests selected for use with this study population. Executive functioning was evaluated as follows:

- **Digit Span**: *Forward Digit Span and Backward Digit Span* are tests of attention and working memory, where the number of digits repeated correctly is noted. They measure the efficiency of attention (freedom from distractibility) and mental tracking (how many bits of information a person can attend to at once and repeat in reverse order), respectively.

- **Wisconsin Card Sorting Test** (WCST, Heaton, 1981) measures abstracting ability and set-shifting capacity. Important measures are the number of perseverative responses (perseverative errors) and the number of categories achieved (a measure of set-shifting).

- **Trails Making Test- Part B** is a test that requires scanning and visuomotor tracking, divided attention and cognitive flexibility. Trails B tests the ability to shift

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attention during an ongoing activity and to allocate attention to more than one type of stimulus at a time (letters and numbers) (Spreen & Strauss, 1991).

- **Phonemic Fluency** (FAS): tests verbal fluency and the ability to initiate and sustain responses. In this task as many words as possible beginning with the letters F, A, or S had to be generated within 60s. Participants were instructed not to repeat the same words, use pronouns, or continuations of the same words.

- **Dementia Rating Scale** (initiation/perseveration and conceptualization) (Mattis, 1976): the initiation and perseveration sub-test scores and the conceptualization subtest score were included.

**MRI**

All brain images were acquired using a 1.5 T Signa MR imager (GE Medical systems, Milwaukee, WI). Three image sets were acquired in the same imaging session: T1-weighted (axial 3D SPGR with 5ms TE, 35ms TR, 35° flip angle, 1 NEX, 22 x 16.5 cm FOV, 0.859 x 0.859mm in-plane resolution, and 1.2 to 1.4mm slice thickness), proton-density (PD) and T2-weighted images (interleaved axial spin echo, with TEs of 30 and 80 ms, 3s TR, 0.5 NEX, 20 x 20cm FOV, 0.781 x 0.781mm in-plane resolution, and 3mm slice thickness).

**SPECT Scans and Regional Perfusion Ratios**

SPECT imaging was performed using a triple-head gamma camera (Prism 3000XP; Phillips Medical Systems Inc, Cleveland, Ohio), a minimum of 30 min and a maximum of 120 min after injection of 20 mCi (740 MBq) of the radiopharmaceutical Technetium-99m ethyl cysteinate dimer (99mTc-ECD). Each view consisted of a 128x128 pixel image with a typical reconstructed image resolution of 9.7 mm full-width at half-maximum. Total imaging time was 19 min. Reconstruction was performed using a

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ramp-filtered back-projection algorithm followed by a 3-dimensional restoration post-filter (Wiener filter, multiplier 1.0). Ellipses were fit to the approximate location of the outline of the head in each transaxial image and a calculated attenuation correction applied (Chang, 1978). Reconstructed images were co-registered to a region-of-interest SPECT template based on an annotated MRI in space similar to the dimensions of the SPECT template (Lobaugh, Caldwell, Black, Leibovitch, & Swartz, 2000). Because the cerebellum is frequently used to normalize SPECT counts (Pickut et al., 1999; Talbot, Lloyd, Snowden, Neary, & Testa, 1994), mean perfusion in regions of interest (ROI) was referenced to the cerebellum to provide semi-quantitative measures of regional cerebral blood flow (rCBF).

**Regions of Interest**

Dorsolateral prefrontal and anterior cingulate cortices were hypothesized to be the regions most adversely affected by SH. The dorsolateral prefrontal cortex ROIs included the middle frontal gyrus (Brodman’s area [BA] 46 and 9) and the anterior region of the superior frontal gyrus (BA 8/9). The anterior cingulate ROIs encompassed BA 24/32/33 (Figure 1). Three additional ROIs, were assigned as control regions to investigate perfusion in areas typically affected in AD: posterior cingulate (BA 23/31), angular gyrus (BA 39), and inferior parietal (BA 40) (Callen, Black, & Caldwell, 2002; Holman, Johnson, Gerada, Carvalho, & Satlin, 1992).

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Figure 1: Frontal regions as defined by the Single - Photon Emission Computed Tomography (SPECT) region-of-interest template. Black-Dorsolateral Prefrontal Gyrus (Brodmann’s Area 9/46), White- Anterior Cingulate Cortex (Brodmann’s Area 24/32/33).

Subcortical Hyperintensities Rating System

A consensus derived rating scale developed under the auspices of the European Task Force in Age-Related White Matter Changes was used to rate SH severity (ARWMC (Wardlaw et al., 2004; Pantoni et al., 2002; Mantyla et al., 1997; Wahlund et al., 2001)). The ARWMC was selected because it was designed to address some of the

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reliability problems encountered with previous scales (Wardlaw et al., 2004). Details of this scale have been published elsewhere (Wahlund et al., 2001), and the ARWMC has shown promise in a recent study of SH progression in AD (de Leeuw et al., 2005).

Severity of SH were rated on PD and T2-weighted MR images in five regions in each hemisphere: frontal, parieto-occipital, temporal, basal ganglia and infratentorial. SH were designated if they were hyperintense on both PD-, T2-weighted images, and were \( \geq 5\text{mm} \) in diameter. Severity was graded from 0 (none) to 3 (severe) based on the appearance of the SH (see below). A measure of global severity was derived by summing the ratings for the 5 regions. Unlike other scales (Fazekas et al., 1987; Scheltens et al., 1992), the ARWMC does not distinguish between periventricular and deep white hyperintensities.

The ARWMC scale does not provide guidelines for distinguishing between levels of global severity of SH. The following criteria were applied to classify patients into Severe SH, Moderate SH, or No SH subgroups: severe SH patients had extensive periventricular or deep white matter hyperintensities (visual rating scale score of 3 (diffuse involvement) in two areas and \( \geq 2 \) (beginning confluence) in two other areas, (Figure 2C). Moderate SH patients had a score of 1 (focal hyperintensities) in more than one area or a score of two (beginning confluence) in any area (Figure 2b). Subjects with minimal (i.e. no more than one small focal, non-lacunar hyperintensity) or no SH were designated as having No SH (Figure 2A).

Two additional measures were utilized to complement the ARWMC data. SH involving cholinergic projections are associated with selective cognitive deficits in cognitively impaired patients (Swartz et al., 2003), and it was hypothesized that

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involvement of these tracts may also affect rCBF in frontal regions. The Cholinergic Pathways HyperIntensities Scale (CHIPS), a recently published MRI visual rating scale for cholinergic tract involvement (Bocti et al., 2005) was applied to quantify SH burden within these specific pathways. The CHIPS scale separates the medial (cingulate gyrus white matter) and lateral (external pathways and claustrum) cholinergic pathways into 10 regions, using major anatomical landmarks on 4 index slices spanning the lateral ventriciles and 3rd ventricle in the axial plane.

Severity of WMH was visually rated on a 3-point scale for each region. Each slice was weighted to account for the decreasing concentration of cholinergic fibers as they project up and fan out in the white matter (maximum score is 50 per hemisphere, with a total of 100 per scan) (Bocti et al., 2005).

Lacunes, defined as small areas (<1.5cm in diameter) in subcortical regions which are hyperintense on T2 and isointense to CSF on T1, are believed to represent cystic infarction (Reed et al., 2004). Their presence may also affect cognition and increase the likelihood of expression of dementia in early AD (Esiri, Nagy, Smith, Barnetson, & Smith, 1999). Although some previous studies have found no significant influence of lacunes on glucose metabolism, cerebral blood flow or neuropsychological test performance (Fein et al., 2000; Mungas et al., 2002), it was still considered important to rule them out as a contributing factor. The total number of lacunes was counted by a neuroradiologist (F.G), blinded to clinical information. Lacunes were differentiated from dilated perivascular spaces by signal characteristics, size and location (Hsu et al., 2002).

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Figure 2: Examples of SH on Axial PD and T2-weighted MR images. Three different severities as classified by the ARWMC scale, (A) No SH (B) Moderate SH (C) Severe SH

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Statistical Analysis

To explore whether the groups differed significantly on cerebrovascular risk factor measures, an analysis of variance (ANOVA) was performed on the total number of CVD risk factors. Categorical variables, such as sex, were compared using Chi-square \( (\chi^2) \) analysis. Correlations between SPECT ROI measures and EF measures were tested with Pearson correlation. Between group differences in SPECT perfusion ratios in the two bilateral ROIs were compared by multivariate analysis of variance (MANOVA). The Bonferroni correction was used for multiple comparisons among the groups. Dunett’s T3 pairwise comparison was used in those cases in which Levene’s test of homogeneity of variance was significant. Power calculations based on the sample data indicated 85% probability of detecting “large” (0.6-effect size) differences. Data analysis was performed using SPSS V.12.0 (SPSS Inc, Chicago, Ill). The level of significance was p<0.05.

RESULTS

Subjects

Data for sixty-three patients met inclusion criteria for this study (26 males and 37 females; mean ± SD age, 76.2 ± 6.3 yr), with a mean educational level of 13.6 ± 3.7yr. The mean ± SD MMSE score across all participants was 23.8 ± 3.8 (range 12-30), indicating mild to moderate AD on average. Groups differed on sex, with more women in the severe and moderate SH groups \( (\chi^2=10.1, \ p<0.01) \). Severe SH participants were more likely to have CVD risk factors than no SH patients \( (\chi^2=13.6, \ p<0.01) \). Characteristics of the participants by SH group are given in Table 1.

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Table 1: **Demographic characteristics of the sample (N=63)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No SH</th>
<th>Moderate SH</th>
<th>Severe SH</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Subjects</td>
<td>20</td>
<td>23</td>
<td>20</td>
<td>ns</td>
</tr>
<tr>
<td>Men/women</td>
<td>14/6</td>
<td>7/16</td>
<td>5/15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age, y</td>
<td>71.8 (6.3)</td>
<td>75.4 (6.3)</td>
<td>78.6 (6.5)</td>
<td>ns</td>
</tr>
<tr>
<td>MMSE</td>
<td>24.0 (3.8)</td>
<td>23.4 (3.7)</td>
<td>24.1 (4.1)</td>
<td>ns</td>
</tr>
<tr>
<td>CVD risk factors</td>
<td>35%</td>
<td>48%</td>
<td>90%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>YOE</td>
<td>14.2 (4.3)</td>
<td>13.5 (3.7)</td>
<td>13.1 (3.2)</td>
<td>ns</td>
</tr>
<tr>
<td>Total ARWMC score</td>
<td>1.1 (0.9)</td>
<td>8.8 (3.7)</td>
<td>16.2 (2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total CHIPS score</td>
<td>n/a</td>
<td>12.4 (11.9)</td>
<td>29.6 (16.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lacunes</td>
<td>n/a</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>No. of subjects with ≥ 1 lacune</td>
<td>n/a</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Mean number of lacunes</td>
<td>n/a</td>
<td>0.91 (2.0)</td>
<td>2.5 (4.7)</td>
<td>ns</td>
</tr>
</tbody>
</table>

χ² and One-way ANOVA group comparisons. Numbers are mean (SD).

YOE= Years of Education; MMSE = Mini-Mental State Examination (Folstein, Folstein, & McHugh, 1975)
ARWMC = Age-related White Matter Changes Scale (Wahlund et al., 2001)
CHIPS = The Cholinergic Pathways HyperIntensities Scale (Bocti et al., 2005)

**Neuropsychological Findings**

Performance on EF measures in the three groups is shown in Table 2. One-way ANOVA revealed a significant group difference on the DRS-conceptualization, with severe and moderate groups scoring lower than the no SH, although only the comparison between the severe and no SH reached significance (F=3.33(2,60)). No other differences were seen on EF. Correlations were investigated between EF scores and regional perfusion ratios in the 4 ROIs for the three SH subgroups. No significant correlations between regional perfusion and EF were found for the severe or moderate SH participants. In the no SH group, DRS-initiation/perseveration score was correlated with

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perfusion in the right dorsolateral prefrontal cortex ($r=0.47$, $p<0.05$). When the correlation coefficients were adjusted for multiple comparisons (Bonferroni), all correlations were under the threshold level of significance.

Table 2: **Mean scores on executive function tests.**

<table>
<thead>
<tr>
<th></th>
<th>No SH (n=20)</th>
<th>Moderate SH (n=23)</th>
<th>Severe SH (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phonemic Fluency (FAS)</strong></td>
<td>27.9 (13.4)</td>
<td>26.0 (13.7)</td>
<td>23.4 (14.8)</td>
</tr>
<tr>
<td><strong>Wisconsin Card-Sorting Task</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Categories</td>
<td>2 (1.2)</td>
<td>1.9 (1.6)</td>
<td>1.2 (1.3)</td>
</tr>
<tr>
<td>No. of perseverations</td>
<td>22.5 (14.5)</td>
<td>17.7 (9.6)</td>
<td>21.0 (10.1)</td>
</tr>
<tr>
<td>Trail Making Test B</td>
<td>6.8 (3.7)</td>
<td>5.3 (2.6)</td>
<td>5.4 (3.3)</td>
</tr>
<tr>
<td>Forward Digit Span</td>
<td>9.0 (2.1)</td>
<td>7.4 (2.1)</td>
<td>7.9 (1.6)</td>
</tr>
<tr>
<td>Backward Digit Span</td>
<td>5.5 (2.5)</td>
<td>5.0 (2.2)</td>
<td>5.7 (2.5)</td>
</tr>
<tr>
<td>Dementia Rating Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initiation/Perseveration</td>
<td>28.5 (5.7)</td>
<td>30.4 (5.4)</td>
<td>28.4 (6.5)</td>
</tr>
<tr>
<td>Conceptualization</td>
<td>35.4 (2.9)</td>
<td>33.1 (3.9)</td>
<td>32.0 (5.6)*</td>
</tr>
<tr>
<td>EXEC-Total Score</td>
<td>37.0 (7.9)</td>
<td>35.0 (7.6)</td>
<td>33.0 (8.7)</td>
</tr>
</tbody>
</table>

* significantly different from No SH

Data are mean (SD).

Higher scores indicate better performance, except for WCST-perseverations.

**ROI Analyses**

Table 3 lists the perfusion ratio values in the two ROIs for the three SH groups.

MANCOVA controlling for gender did not reveal group differences in mean prefrontal perfusion ($F_{(8,114)} = 1.80$). Although the MANCOVA did not reveal an overall difference in perfusion based on severity of SH, exploratory univariate ANOVAs indicated differences related to SH severity in the left ($F_{(2,59)} = 4.20$) and right dorsolateral prefrontal cortex ($F_{(2,59)} = 4.46$). In both these regions, the moderate SH group had lower perfusion than the no SH and severe SH groups, with the greatest differences seen between the no SH group and the moderate group. Figure 3 displays the perfusion

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differences among the three groups. No significant group differences were found for the three posterior control ROIs ($F_{(2,59)} = 0.92$). The relationships between global severity scores in the severe SH and moderate SH groups were also examined. No significant correlations were seen between this measure and SPECT or EF measures, within or across groups.

To investigate additional factors contributing to frontal perfusion, we examined the impact of SH in cholinergic pathways in the moderate and severe SH groups. T-tests on the CHIPS scores revealed overall group differences ($t = -3.94, p<0.001$), with the severe SH group showing greatest involvement of the cholinergic tracts. However, no significant correlations were seen between CHIPS and frontal perfusion, or CHIPS and EF scores in this sample.

Lacunar infarcts were considered as a second possible influence on perfusion and cognition. The mean ± SD total number of lacunes across all participants was $1.22 ± 3.1$. Overall, 15 subjects had at least one lesion that met criteria for lacunar infarct: 9 in the severe SH group and 6 in the moderate SH group. Of those subjects with lacunar infarcts, 6 had lacunes only in the basal ganglia area (including external and internal capsule, caudate, putamen and thalamus), 4 had lacunar infarcts only within the deep white matter, and 5 had both basal ganglia and deep white matter lacunes. The number of participants with lacunes did not significantly differ between the moderate and severe groups ($\chi^2 = 2.65$). Exclusion of these 15 subjects did not have any influence on the relationships found between SH and frontal perfusion or executive function. The full statistical analysis was also repeated on this sub-sample and neither the presence of

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lacunar infarcts nor involvement of the cholinergic pathways influenced perfusion patterns.

The three hypotheses of this study were that the severity of SH would correlate negatively with frontal perfusion ratios, severity of SH would correlate with executive function, and involvement of cholinergic tracts would correlate with frontal perfusion. To our surprise, while the data suggested that severity of SH correlates with executive performance, it did not support the hypotheses related to frontal perfusion.

Table 3. Regional Perfusion Ratios

<table>
<thead>
<tr>
<th></th>
<th>No SH (n=20)</th>
<th>Moderate SH (n=23)</th>
<th>Severe SH (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Cingulate cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.83 (0.10)</td>
<td>0.79 (0.07)</td>
<td>0.79 (0.08)</td>
</tr>
<tr>
<td>Right</td>
<td>0.79 (0.07)</td>
<td>0.76 (0.09)</td>
<td>0.77 (0.09)</td>
</tr>
<tr>
<td>Dorsolateral Prefrontal gyrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.79 (0.06)</td>
<td>0.76 (0.06)</td>
<td>0.79 (0.07)</td>
</tr>
<tr>
<td>Right</td>
<td>0.76 (0.06)</td>
<td>0.72 (0.06)</td>
<td>0.75 (0.05)</td>
</tr>
<tr>
<td>Posterior Cingulate cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.76 (0.08)</td>
<td>0.72 (0.13)</td>
<td>0.75 (0.09)</td>
</tr>
<tr>
<td>Right</td>
<td>0.78 (0.07)</td>
<td>0.73 (0.11)</td>
<td>0.76 (0.09)</td>
</tr>
<tr>
<td>Angular Gyrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.79 (0.12)</td>
<td>0.77 (0.10)</td>
<td>0.79 (0.08)</td>
</tr>
<tr>
<td>Right</td>
<td>0.8 (0.09)</td>
<td>0.76 (0.11)</td>
<td>0.77 (0.09)</td>
</tr>
<tr>
<td>Inferior Parietal cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.75 (0.09)</td>
<td>0.73 (0.8)</td>
<td>0.75 (0.08)</td>
</tr>
<tr>
<td>Right</td>
<td>0.74 (0.07)</td>
<td>0.73 (0.9)</td>
<td>0.79 (0.78)</td>
</tr>
</tbody>
</table>

Numbers are means (SD).

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DISCUSSION

The current study investigated the impact of white matter hyperintensity burden in AD on frontal perfusion. Our hypothesis was that the severity of SH would correlate negatively both with frontal perfusion ratios on SPECT and performance on executive function tasks. In general, the current findings do not support this hypothesis, but rather support previous findings by Ott et al. (1997) showing little correlation between presence of SH and frontal perfusion. Patient groups did not differ in age, education, or dementia severity. Using a SH visual rating scale to classify subjects, subtle disturbances were found in selected frontal lobe functions in patients with large SH burden. Executive function differences were seen between the participants with severe SH and no SH on a

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task that assessed concept formation (identify how items are similar or different). We were unable to discern a statistically significant relationship between global burden of SH on MRI and frontal perfusion as measured by SPECT, unlike one previous study (Starkstein et al., 1996). We did find, however, that moderate SH subjects had slightly lower frontal perfusion ratios in two of the four frontal regions examined.

As expected, based on our classification criteria, subjects in the severe SH group tended to have more diffuse periventricular hyperintensities. SH in the moderate group consisted primarily of focal deep white and basal ganglia hyperintensities even though this classification allowed for SH beginning to show confluence. These two types of SH are not fully separated in the current implementation of the ARWMC scale, but diffuse SH are rated as more severe than focal SH. Our data lend some support to the view that smaller more punctuate lesions found in the deep white matter and basal ganglia may be more detrimental to regional perfusion than larger confluent SH. The trend towards decreased perfusion in the moderate SH group suggests that the location and type of SH may be more important than overall lesion burden. We are currently extending our analysis of SH in those patients to test this idea more formally.

SH may contribute relatively more to cognitive decline in patients who are at an earlier stage of AD pathogenesis. SIVD may have a greater influence on cognitive performance (Tullberg et al., 2004) and frontal metabolism (Esiri et al., 1999) in the earlier stages of AD, compared to more advanced stages, when AD pathology has progressed to the point that it is difficult to distinguish the specific effects of SIVD. The current study differed from many previous studies in that the groups were well matched on a cognitive measure of disease-severity (MMSE score, age, YOE). However, it is

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possible that the three groups differed on pathologic stage of AD. As many of these patients are being followed longitudinally, we will be able to determine whether the patterns of their neuropsychological deficits change and are accompanied by changes in the location, size and types of SH, and frontal perfusion.

Studies that have looked specifically at the relation between SH and cerebral perfusion or cortical metabolism have shown inconsistent results. Some report no relationship between SH and perfusion in patients with dementia (Sabri et al., 1999; Ott et al., 1997), while others suggest SH negatively affect both global and regional metabolism (Reed et al., 2004; Herholz et al., 1990). Decreased frontal lobe metabolism has been reported in cognitively normal individuals with significant periventricular SH (DeCarli et al., 1995), but this relationship has not yet been found in studies of dementia or cognitively impaired patients (DeCarli et al., 1996; Reed et al., 2004). Herholz et al. (1990) proposed that only large confluent lesions constituted clinically-relevant SIVD that would likely influence cerebral blood flow. Likewise, in a group of healthy elderly, only SH that encompassed more than 0.5% of intracranial volume were associated with decreased cognition and frontal metabolism (DeCarli et al., 1995). In the current study, the amount of SH as rated on a severity scale did not appear to be associated with a decline in frontal perfusion, as no strong relationships were found across the sample or within subgroups.

Several additional factors that may influence cognition and cerebral functioning were examined in this study. The potential involvement of hyperintensities located in the cholinergic pathways, which correlate specifically to the dysexecutive syndrome often seen in individuals with SH (Swartz et al., 2003) did not explain the findings. Other

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studies have suggested that lacunar infarcts may contribute to cognitive and functional
decline in SIVD and AD (Snowdon et al., 1997; Reed et al., 2004). The presence of
silent brain infarcts in healthy elderly increases the risk of dementia and predicts a faster
decline in cognitive function (Vermeer et al., 2003). Our study excluded individuals with
a history of stroke and strategic infarct (unilateral or bilateral infarct in strategic area –
thalamus or hippocampus) (O'Brien, 2006), which reduced the overall number of patients
with lacunes and the number of lacunes per patient in our sample. As the number of
lacunar infarcts seen in the moderate and severe groups was not large, this sample may
not reflect the typical effect that lacunes may have on function.

Some evidence suggests that “pure” AD and “pure” Vascular Dementia are
relatively uncommon and that AD in combination with SIVD may be more the more
prevalent condition (Kalaria, 1999). A recent large population study examined the extent
of SH on MRI in a sample of elderly participants and found that very few (<5%) had no
or barely detectable changes on MRI (Kuller et al., 2004). Although, it was difficult to
identify cases of “pure” AD patients, that is, patients who had no SH, we were able to
identify a reasonable sample size given the low prevalence of this condition in the
general population.

The visual assessment of SH, though straightforward, is not ideal. Existing rating
scales vary in the morphological description of SH, the brain regions that are included,
and do not agree on how to weight the anatomical distribution of the hyperintensities
(e.g. perventricular vs. deep white matter SH). Furthermore, the use of an ordinal visual
rating scale with a predefined maximum score as a method of categorizing severity of
SH could potentially introduce groupings that may not best define the population of

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patients. What is needed to better understand the relationship of SH to cognition and functional brain measures is quantitative analyses of lesion volume as a continuous measure within different anatomical regions. Such studies will also provide more sensitive measures to follow progress of SH patterns and to understand better whether executive and perfusion deficits worsen in parallel with SH progression (Admiraal-Behloul et al., 2005).
REFERENCE LIST


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DeCarli, C., Murphy, D. G., Tranh, M., Grady, C. L., Haxby, J. V., Gillette, J. A. et al. (1995). The effect of white matter hyperintensity volume on brain structure,


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CHAPTER III-A
WHITE MATTER HYPERINTENSITY TOPOGRAPHY IN ALZHEIMER’S DISEASE- CORRELATIONS WITH COGNITION

INTRODUCTION

In structural neuroimaging, automatic segmentation algorithms can be used to obtain volumetric information derived from voxel intensity differences with a set of multi-modal magnetic resonance imaging (MRI) brain acquisitions. Segmentation techniques vary in approach but generally provide the same information - tissue volumes for grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) (Kovacevic et al., 2002; DeCarli et al., 1992). Due to increasing awareness of the presence of white matter hyperintensities (WMH) in both normal aging and degenerative diseases, some studies have aimed to separately measure the exact volume and topography of WMH (Admiraal-Behloul et al., 2005; DeCarli, Fletcher, Ramey, Harvey, & Jagust, 2004; Wu et al., 2006). Volumetric measurement of WMH uses computerized tissue segmentation procedures that are specifically designed to quantify focal or diffuse hyperintense lesions frequently seen in the cerebral white matter and deep nuclei. These studies have provided new insight into the extent and regional distribution of WMH (Wen et al., 2004); however, debate persists as to the anatomic distinctions (deep white and periventricular regions) of WMH and their relationships with both brain atrophy and cognitive impairment (DeCarli et al., 2004; Sachdev & Wen, 2005).

In a previously reported cross-sectional sample from the Sunnybrook Dementia study, (Levy-Cooperman et al., 2007a), patients with a range of severity of WMH were classified using a visual rating scale and compared on measures of cognition and measures of brain perfusion. This study revealed small differences between patients with
different severities of WMH on tests of conceptualization but not on regional brain perfusion. While rating scales are informative, the use of an ordinal visual rating scale with a predefined maximum score as the method of categorizing severity of WMH may have introduced groupings that did not optimally define the Alzheimer’s disease (AD) population. In the current study, therefore, semi-automatic tissue classification techniques were employed to further characterize the same AD sample using continuous volumetric measures.

Quantitative studies typically distinguish WMH by their subcortical location into two categories, periventricular hyperintensities (PVH) and deep white hyperintensities (DWH) (DeCarli et al., 2004; DeCarli et al., 2005; Yoshita et al., 2005; Yoshita et al., 2006). PVH occur in those areas surrounding the cerebral ventricles while DWH are patchy or focal areas of hyperintensity distinct from PVH further out toward the cortex and also involving the basal ganglia and thalamus. According to a few studies, these lesion types are highly correlated in their anatomical location (Swartz, 2003), leading one group to suggest that any distinction based on anatomical location may be arbitrary (Swartz, 2003; DeCarli et al., 2004). Other researchers suggest that the categorical distinction between the two subtypes is necessary as they may have neuropathological differences (Sachdev et al., 2005).

Many AD patients also have concomitant lacunar infarcts in addition to or within their WMH. Lacunar infarcts have been examined previously in elderly controls and dementia populations; however, the relationship between lacunes and both AD and cerebrovascular disease (CVD) is still not understood. Some studies found that dementia in individuals with CVD correlates best with hippocampal and grey matter atrophy,
rather than any measure of lacunes (Fein et al., 2000; Mungas et al., 2002), while other studies have found a relationship between the presence of lacunar infarcts and decreased metabolic rates in the dorsolateral prefrontal cortex (Reed et al., 2004) and also a decline in executive function (Mungas et al., 2005).

Pathology studies have also reported that the risk of cognitive decline in patients with AD is amplified by the presence of cerebrovascular pathology, such as stroke, transient ischemic attacks and WMH. Still, a relationship between total volume of WMH and cognitive impairment, although thought to correlate best with executive dysfunction, has not been consistently replicated (Tupler, Coffey, Logue, Djang, & Fagan, 1992; Boone et al., 1992; Reed et al., 2007).

The current study was designed to quantify WMH in a group of mild to moderate AD patients with varying severity of WMH. The aim was to 1) examine the regional distribution of WMH in 13 bilateral brain regions for PVH, DWH and lacunar infarcts treated as distinct subtypes of WMH, 2) determine whether WMH volumes correlated with performance on cognitive tests in our AD sample. We also sought to 3) investigate the impact of cerebrovascular risk factors on WMH.

METHODS

Participants

Participants were volunteers of the Sunnybrook Dementia Study, recruited from the Cognitive Neurology Clinic at Sunnybrook Health Sciences Centre, a University of Toronto academic healthcare institution. All patients received a comprehensive clinical evaluation, including detailed medical history, neurological examination, routine laboratory investigation and neuropsychological testing with a standardized test battery. Individuals were excluded if the MRI and neuropsychological testing were separated by
more than 12 weeks, or if the MRI scans were technically inadequate for volumetric analysis. All subjects were greater than 60 years of age and had to be fluent in English to be included. Secondary causes of dementia (other than subcortical vascular disease), concomitant neurological or psychiatric illnesses, or significant co-morbid systemic illness were exclusionary. This protocol was reviewed and approved by the institutional Research Ethics Board, and written informed consent was obtained from all subjects or their substitute decision makers.

In the current study, patients had the historical profile typical of AD with insidious onset of short term memory loss and gradual decline of function and cognition. 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 et al., 1984), and Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (American Psychological Association, 1994) criteria for dementia. Diagnosis was reached by consensus of two clinicians based on these criteria, including one year of observation. Demographic information was collected on all participants including age at time of magnetic resonance scan, number of years of education, sex, duration of cognitive impairment and presence of cerebrovascular risk factors. In this sample, the diagnosis of “Possible” AD was based only on the presence of sufficient subcortical CVD to contribute to dementia, but patients with extensive WMH did not meet criteria for Vascular Dementia (VaD); that is, patients were excluded if they met the National Institute for Neurological Disorders and Strokes (NINDS) - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) criteria for possible or probable VaD, which requires focal
neurological signs in addition to imaging evidence of CVD. Patients with possible AD due to other causes or focal memory deficit alone were not included. Presence of silent cortical strokes on MRI was also exclusionary.

**Cerebrovascular Risk Factors**

The presence or absence of vascular risk factors was systematically assessed in subject histories, as well as a review of pertinent medical records, to create a vascular risk factor composite score ranging from 0-10 and reported as a percentage. The Vascular Risk factors scale assigned scores for treated (2 pts) or untreated hypertension (4pts), treated (1 pt) or untreated hyperlipidemia (2pts), diabetes (2.5 pts), and currently (1.5pts) or quit smoking (0.5pts). Information was also collected on history of stroke, transient ischemic attack (TIA), myocardial infarction, angina, and peripheral vascular disease, and on medication history (e.g., use of anticonvulsants, antidepressants, antipsychotic, antithrombotics, antihypertensive, ACE inhibitors, beta-blockers, cognitive enhancers, diabetes treatments, hormone replacement therapy, lipid lowering medication, NSAIDS, thyroid treatment). Altogether, four patients were taking PRN benzodiazepine (3 Lorazepam, 1 Alprazolam) and two were on small doses of Risperidone.

**Neurobehavioral Assessment**

Cognitive function was assessed with a battery of neuropsychological tests selected for use with this study population. Specific tests were chosen with the intention of: a) providing an assessment of general cognitive function and b) to index impairments common in AD in specific cognitive domains sampled to reflect dysfunction associated with particular brain regions, the following tests were included:

- *Dementia Rating Scale* (Mattis, 1976): This was used as a global measure of
cognitive function. It also generates subset scores for attention, initiation and perseveration, conceptualization, construction and verbal and non-verbal short term memory.

- **Digit Span**: *Forward Digit Span and Backward Digit Span* are tests of attention and working memory, where the number of digits repeated correctly is noted. They measure the efficiency of attention (freedom from distractibility) and mental tracking (how many bits of information a person can attend to at once and repeat in reverse order), respectively.

- **Wisconsin Card Sorting Test** – 64 item version (WCST), (Heaton, 1981) measures abstracting ability and set-shifting capacity. Key measures used in this study were the number of perseverative responses (perseverative errors- a measure of mental flexibility) and the number of categories achieved (a measure of abstraction and set-shifting).

- **Trails Making Test Part A & B**- is a test that probes visuomotor tracking, divided attention and cognitive flexibility. Trails-A requires an individual to draw lines sequentially connecting 25 encircled numbers distributed on a sheet of paper. Task requirements are similar for Trails-B except the person must alternate between numbers and letters in serial order (e.g., 1, A, 2, B, 3, C, etc.). The score on each part represents the amount of time required to complete the task. Trails B tests the ability to shift attention during an ongoing activity and to allocate attention to more than one type of stimulus at a time (Spreen et al., 1991).

- **Phonemic Fluency (FAS)**: tests verbal fluency and the ability to initiate and sustain responses. In this task as many words as possible beginning with the letters F, A, or S had to be generated within one minute for each letter. Participants were instructed not to
repeat the same words, use pronouns, or continuations of the same words (Canning et al., 2004).

- **Semantic Fluency (SF)**: tests semantic fluency and the ability to initiate and sustain responses. In this task as many words as possible in the category animals had to be generated within 60s. Participants were instructed not to repeat the same words (Canning et al., 2004).

- **Benton Line Orientation** (Benton A.L., Hamsher, Varney, & Spreen, 1983): tests visuospatial orientation and attention. This task requires the identification of lines matching a target with an array of differently oriented lines.

- **Rey Osterrieth Complex Figure –Copy** (Osterrieth, 1944): tests perceptual organization, visual/spatial perception and construction. Patients are asked to copy a complex geometric stimulus on a blank sheet of paper.

- **Boston Naming Test (30-item)** (Kaplan, Goodglass, & Weintraub, 1978) – tests language and object naming. This is a picture-naming vocabulary test in which patients are asked to name a series of line drawings.

- **California Verbal Learning Test-Aquisition Sub-Score** (Delis D.C., Kramer, Kaplan, & Ober, 1987) – tests verbal learning and memory. Patients are required to learn semantically related words from a 16-item list over a series of 5 trials.

**MR Image Acquisition**

Magnetic resonance images were acquired on a 1.5 Tesla Signa scanner (GE Medical Systems, Milwaukee, Wisconsin) and complied with the consensus panel imaging recommendations on Vascular Cognitive Impairment (Hachinski et al., 2006b). Three image sets were acquired in the same imaging session: T1-weighted (an axial 3D SPGR with 5ms TE, 35ms TR, 1 NEX, 35° flip angle, 22 x 16.5 cm FOV, 0.859 x 0.859
mm in-plane resolution, and 1.2 to 1.4mm slice thickness depending on head size),
proton density (PD) and T2-weighted (interleaved axial dual-echo spin echo with TEs of
30 and 80 ms, 3s TR, 0.5 NEX, 20 x 20cm FOV, 0.781 x 0.781mm in-plane resolution,
and 3mm slice thickness).

**MR Image Post-processing**

Brain extraction and automated tissue segmentation was accomplished using a
modified version of previously described methods (Kovacevic et al., 2002). PD and T2-
weighted images were coregistered to the T1-weighted image using automated image
registration software (AIR, v.5.2 23). The PD/T2 images were used to extract brain and
subdural CSF and used to mask the T1. The masked T1 was segmented using a T1-based
segmentation, which is a robust and reliable tissue segmentation protocol optimized for
elderly and AD populations (Phantom: coefficient of agreement=0.97, Scan-Rescan
differences: Global < 1% of total intracranial volume (TIV), Local < 0.15% of TIV)
(Kovacevic et al., 2002).

Brain region parcellation was accomplished using a modified version of our
previously described methods for semi-automated brain region extraction (SABRE)
(Dade et al., 2004). SABRE is a highly reliable method which parcellates each individual
brain into 26 brain regions proportional to individual head sizes (Inter-class Correlation
range: 0.97-0.99 for individual tissue classes in each region). A set of easily identified
landmarks were traced on the masked T1 images using the 3D rendering and region of
interest (ROI) modules in the ANALYZE software package (Biomedical Imaging
Resource, Mayo foundation, Rochester, MN, USA): the central sulcus, sylvian fissure,
parieto-occipital sulcus, anterior commissure and posterior commissure. An in-house
program combined these landmarks with the Talairach proportional grid system
(Talairach J, 1988) to generate individualized maps of 13 lobular regions in each hemisphere. Figure 1 shows a map of the 13 regions which consisted of: superior, middle, and inferior frontal, superior, middle, and inferior medial frontal, superior and inferior parietal, anterior and posterior temporal, anterior and posterior basal ganglia and thalamus, and occipital regions.

Figure 1: Three-dimensional surface CSF eroded image and axial view with different colors representing SABRE parcellated brain regions.

White matter hyperintensity segmentation was accomplished using a previously described method called Lesion Explorer (Quddus et al., 2004; Ramirez, 2005). This semi-automated procedure used an intensity cut-off based on a weighting of the PD and T2 images to define putative WMH. A 3-dimensional voxel dilation procedure was applied to the T1-segmentation's ventricular volumes using a 2 voxel dilation factor in ANALYZE. This was to account for partial volume effects and for the elimination of "pencil thin" periventricular hyperintensities which are believed to reflect T2-weighted
flow artifacts from CSF. The output was then manually edited by a trained operator who accepted relevant hyperintensities (based on concurrent evaluation of PD and T2 weighted images) to generate final lesion volumes (ICC range for 26 SABRE brain regions: 0.96-0.99). Lobar volumes of different tissue types were normalized to the supratentorial TIV and expressed as a percentage. When examining WMH distribution by brain region, WMH volumes were expressed as mean percent of the three tissue types contributing to a specific region of interest (GM + WM + CSF- ventricular and sulcal= Total Regional Volume), this was referred to as Total Regional Volume (TRV). PVH were identified as those hyperintensities that connected to the ventricle in three-dimensional space. DWH in the current study only included those hyperintensities that were discrete and not connected to the ventricle, including those in the deep nuclei.

**Lacune Measurements**

Using the T1 segmentation, an additional subcategory of WMH containing fluid-filled cysts, less than 2.0cm³ was included as lacunar volumes. Lacune volumes were reported as a ratio of TIV and also as a ratio of TRV and were also separated based on location within either the DWH or the PVH. An overall count of discrete lacunes was also reported. Lacunes were distinguished from Virchow-Robins spaces during the manual hyperintensity selection using the T1-image for confirmation. Virchow-Robin spaces were defined as isointense to CSF on T1, T2, and PD sequences (Barkhof, 2004). Therefore the output of the lesion segmentation was 3 subtypes: PVH, DWH and lacunes in 26 regions including basal ganglia and thalamus.

**Statistical Analysis**

Data analysis was performed using SPSS software version 12.0 (SPSS Inc, Chicago, Ill). CVD risk factor differences were examined using independent-sample \( t \)-
test analysis for continuous variables and chi-square analysis for categorical variables.

Pearson correlation analyses were used to investigate relationships between brain volume measures and performance on neuropsychological tests. The level of significance was set at $p<0.05$.

RESULTS

Descriptive Statistics

Sixty-three probable or possible AD patients were included in this study. Four patients were excluded due to poor scan quality for a total of 59 subjects in the final sample. Demographic information and tissue volumes for the sample are summarized in Table 1. The average age of the participants was 76 ± 6.3 years (range, 62-90). Approximately 41% of the sample was male. The average age did not differ significantly by gender (76.2 ± 5.8 for men and 76.1 ± 6.7 for women). Further sex differences are presented in chapter III-B. Hyperintense signals on T2- and PD-weighted images were present in all 59 subjects, but their extent and distribution varied considerably. As expected, participants with CVD risk factors (n=32, 54%) had a significantly higher score on the vascular risk factor scale ($t = -3.9, P<0.01$) (Table 2), and also had a significantly greater volume of DWH ($F = 5.65, P<0.05$), PVH ($F = 9.07, P<0.01$), and vCSF ($F = 23.8, P<0.001$), as well as smaller WM volumes ($F = 10.8, P<0.01$). No significant differences were seen in total volume of GM or lacunar infarcts in those patients with or without CVD risk factors. Correlation analysis revealed a positive relationship between total PVH volume and CVD risk factor score ($r = 0.28, p<0.05$), but not DWH or lacunar infarct volumes.
<table>
<thead>
<tr>
<th></th>
<th>Total (N=59)</th>
<th>Male (n=24)</th>
<th>Female (n=35)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>76.1 (6.3)</td>
<td>76.2 (5.8)</td>
<td>76.1 (6.7)</td>
<td>t = 0.03, P = 0.97</td>
</tr>
<tr>
<td><strong>Mini-Mental Status Exam</strong></td>
<td>24.1 (3.7)</td>
<td>24.5 (3.7)</td>
<td>23.9 (3.8)</td>
<td>t = 0.60, P = 0.55</td>
</tr>
<tr>
<td><strong>Years of Education</strong></td>
<td>13.4 (3.8)</td>
<td>14.2 (4.0)</td>
<td>12.9 (3.5)</td>
<td>t = 1.30, P = 0.20</td>
</tr>
<tr>
<td><strong>CVD Risk Factor (%)</strong></td>
<td>14.2 (14.6)</td>
<td>16.5 (16.2)</td>
<td>12.6 (13.4)</td>
<td>t = 0.98, P = 0.33</td>
</tr>
<tr>
<td><strong>CGM</strong></td>
<td>42.4 (2.5)</td>
<td>41.3 (2.2)</td>
<td>43.2 (2.4)</td>
<td>F = 9.54, P = 0.003</td>
</tr>
<tr>
<td><strong>WM</strong></td>
<td>29.5 (3.0)</td>
<td>30.1 (3.3)</td>
<td>29.1 (2.8)</td>
<td>F = 1.53, P = 0.22</td>
</tr>
<tr>
<td><strong>sCSF</strong></td>
<td>21.9 (3.5)</td>
<td>23.1 (3.7)</td>
<td>21.1 (3.2)</td>
<td>F = 4.81, P = 0.03</td>
</tr>
<tr>
<td><strong>vCSF</strong></td>
<td>4.64 (1.8)</td>
<td>4.37 (1.3)</td>
<td>4.82 (2.1)</td>
<td>F = 0.89, P = 0.35</td>
</tr>
<tr>
<td><strong>DWMH</strong></td>
<td>0.11 (0.17)</td>
<td>0.06 (0.08)</td>
<td>0.15 (0.20)</td>
<td>F = 4.78, P = 0.03</td>
</tr>
<tr>
<td><strong>PVWMH</strong></td>
<td>1.4 (1.6)</td>
<td>1.1 (1.5)</td>
<td>1.6 (1.7)</td>
<td>F = 1.53, P = 0.05</td>
</tr>
<tr>
<td><strong>Lacunes</strong></td>
<td>1.5 (3.0)</td>
<td>0.97 (1.5)</td>
<td>1.8 (3.6)</td>
<td>F = 1.11, P = 0.29</td>
</tr>
</tbody>
</table>

Data presented as mean (SD).
* MRI volume measures are expressed as % mean (SD) of TIV.
**Lacunar Infarct volumes are expressed as % mean (SD) of total brain WMH.
WM= white matter; CGM=cortical grey matter; vCSF= Ventricular Cerebrospinal fluid; sCSF= Sulcal Cerebrospinal Fluid; DWMH= deep white matter hyperintensities; PVWMH= periventricular white matter hyperintensities.
Table 2. Mean total brain MRI segmentation and WMH volumes comparing subjects with and without CVD risk factors (N=59)

<table>
<thead>
<tr>
<th></th>
<th>CVD risk factors (n=32)</th>
<th>No CVD risk factors (n=27)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>76.9 (6.0)</td>
<td>75.2 (6.7)</td>
<td>1.05</td>
<td>0.31</td>
</tr>
<tr>
<td>CVD Score</td>
<td>2.0 (1.6)</td>
<td>0.71 (0.89)</td>
<td>13.9</td>
<td>0.001</td>
</tr>
<tr>
<td>CGM*</td>
<td>42.5 (2.5)</td>
<td>42.4 (2.6)</td>
<td>0.13</td>
<td>0.91</td>
</tr>
<tr>
<td>WM*</td>
<td>28.4 (2.8)</td>
<td>30.9 (2.8)</td>
<td>10.8</td>
<td>0.002</td>
</tr>
<tr>
<td>sCSF*</td>
<td>21.4 (3.4)</td>
<td>22.4 (3.6)</td>
<td>1.01</td>
<td>0.32</td>
</tr>
<tr>
<td>vCSF*</td>
<td>5.5 (1.9)</td>
<td>3.6 (1.0)</td>
<td>23.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DWH*</td>
<td>0.16 (0.21)</td>
<td>0.06 (0.08)</td>
<td>5.65</td>
<td>0.02</td>
</tr>
<tr>
<td>PVH*</td>
<td>2.0 (1.9)</td>
<td>0.76 (0.8)</td>
<td>9.07</td>
<td>0.004</td>
</tr>
<tr>
<td>Lacunes</td>
<td>1.8 (3.8)</td>
<td>1.0 (1.5)</td>
<td>1.2</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Data presented as means (SD).
* MRI volume measures are expressed as % mean (SD) of TIV.
**Lacunar Infarct volumes are expressed as % mean (SD) of total brain WMH. WM = white matter; CGM = cortical grey matter; VCSF = ventricular CSF; DWH = deep white matter hyperintensities; PVH = periventricular white matter hyperintensities

Distribution of WMH by Brain Region

Hemispheric comparisons revealed no difference in total WMH, PVH or DWH; therefore, right and left hemispheres were combined. The mean ± SD volume of total WMH was 18.2 ± 20.2 cm³ per subject, which represents approximately 1.5% ± 1.7 of the TIV and 5.6% ± 6.7 of the total white matter. WMH were further categorized into DWH and PVH. The mean volume of total PVH was 16.9 ± 19.4 cm³ which represented 1.4% ± 1.6 of the TIV, while DWH accounted for a much smaller total volume with a mean of 1.3 ± 2.1 cm³ and 0.11% ± 0.17 of TIV. The average number of discrete DWH per individual was 33.7 ± 32.8 with an average volume of 0.03 cm³ per hyperintensity. The
average number of discrete PVH was $5.7 \pm 3.1$ with a mean volume per discrete PVH of $16.9\text{cm}^3$. The majority of WMH were found in the frontal and parietal regions (see Figure 2 and 3), with the middle frontal and inferior parietal ROIs accounting for 29% ($5.2\text{cm}^3$) and 31% ($6.5\text{cm}^3$) of total WMH, respectively. The medial middle frontal and occipital regions each accounted, approximately, for an additional 11% of total WMH volume. All of the patients had hyperintensities in the periventricular region, and 95% had hyperintensities in the deep white matter. The majority of WMH were found in the periventricular region (90.3%), with only 9% of total WMH found in the deep white matter (see figure 2 and 3). The brain regions most affected were similar for both DWH and PVH. PVH were found most frequent and voluminous in the following areas (for which left and right volumes were combined and expressed as percentage relative to the size of the brain region): inferior parietal (3.3%), middle frontal (3.2%), medial middle frontal (1.7%), and occipital (1.1%). DWH were also found, but to a much smaller extent, in the inferior parietal (0.17%), middle frontal (0.2%) and also in the medial superior frontal (0.23%) regions.
Figure 2: WMH volume of 13 brain regions total WMH, PVH, and DWH. The majority of hyperintensities were found in the periventricular region, defined in this study as a hyperintensity which made contact with the edge of the ventricle when examined three-dimensionally. The middle frontal and inferior parietal regions had the largest volume of hyperintensities for both DWH and PVH. SF- Superior Frontal; MF-Middle Frontal; If- Inferior Frontal; MIF- Medial Inferior Frontal; MSF- Medial Superior Frontal; MMF- Medial Middle Frontal; SP- Superior Parietal; IP-Inferior Parietal; O-Occipital; AT-Anterior Temporal; PT- Posterior Temporal; AB-Anterior Basal Ganglia/Thalamus; PB- Posterior Basal Ganglia/Thalamus.
Figure 3: Regional distribution of WMH (%TRV). Middle frontal and inferior parietal regions had largest WMH volumes accounting for an average of 29% and 31% of total WMH, respectively. SF- Superior Frontal; MF- Middle Frontal; If- Inferior Frontal; MIF- Medial Inferior Frontal; MSF- Medial Superior Frontal; MMF- Medial Middle Frontal; SP- Superior Parietal; IP-Inferior Parietal; O-Occipital; AT-Anterior Temporal; PT- Posterior Temporal; AB-Anterior Basal Ganglia/Thalamus; PB- Posterior Basal Ganglia/Thalamus

Volumetric Measures of Lacunar Infarcts

Lacunar infarcts accounted for 1.6 % of total WMH volume. Information on the number of discrete lacunes and the average volume of discrete lacunes is presented in Table 3. In brief, 86% (n=51) of the sample had at least one lacunar infarct; 71% (n=42) had at least one lacune in the deep white matter; and 69% (n=41) had at least one lacune within the periventricular white matter. The mean number of total brain lacunar infarcts was 5.5 (range 0-22), with a mean of 4 (range 0-20) lacunar infarcts found within the DWH, and a mean of 1.5 (range 0-6) lacunes within the PVH. The average size of
individual lacunes found within the deep white matter was 0.004cm$^3$, and the mean overall volume of all lacunes found within DWH was 0.02cm$^3$ accounting for 1.6% of total DWH. The average size of individual lacunes found within the periventricular region was 0.07cm$^3$ and the mean total volume of lacunes found within PVH was 0.24cm$^3$ accounting for 1.4% of total PVH. The regional distribution of lacunar infarcts as a percent of TRV is presented in Figure 4, with the largest volume of lacunes found in the frontal and anterior basal ganglia/thalamus regions. When lacunes were investigated relative to total WMH in a given region, the highest ratio of lacunar infarct to regional WMH volume was found in the posterior basal ganglia and thalamus region accounting for 4.7% of that region’s total WMH volume, but only 1.4% of whole brain lacune volume. The occipital and anterior temporal regions also had a larger ratio of lacune to WMH with lacunes accounting for 3.2% and 3.4% of regional WMH volume, respectively. These two regions combined accounted for 11.7% of the total brain lacunar volume. Lacune volume was further investigated by examining the regional distribution of lacunes found within the DWH and PVH. This information is presented in Figure 5. In general, lacunar infarcts were much larger when located within PVH, but were fewer in total count. Lacunes found within the DWH had smaller volumes but were more numerous.
Table 3 Mean total brain, PVH and DWH discrete lacune counts and volumes.

<table>
<thead>
<tr>
<th></th>
<th>PVH</th>
<th>DWH</th>
<th>Whole Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrete Lacune Count-</td>
<td>Mean (Range)</td>
<td>1.5 (0-6)</td>
<td>4 (0-20)</td>
</tr>
<tr>
<td>Discrete Lacune Volume -</td>
<td>Mean (SD)</td>
<td>0.07 (0.2)</td>
<td>0.004 (0.005)</td>
</tr>
<tr>
<td>Total Brain Lacune</td>
<td>Mean (SD)</td>
<td>0.24 (0.5)</td>
<td>0.02 (0.03)</td>
</tr>
</tbody>
</table>

* MRI volumes are presented in cm$^3$.
DWH= deep white matter hyperintensities; PVH= periventricular white matter hyperintensities

Figure 4: Regional distribution of lacunar infarcts (% TRV)
The largest ratio of lacunes per TRV was found in the middle frontal (0.9%), Medial Middle Frontal (0.05%) and anterior Basal Ganglia/Thalamus (0.03%). SF- Superior Frontal; MF- Middle Frontal; IF- Inferior Frontal; MIF- Medial Inferior Frontal; MSF- Medial Superior Frontal; MMF- Medial Middle Frontal; SP- Superior Parietal; IP-Inferior Parietal; O-Occipital; AT-Anterior Temporal; PT- Posterior Temporal; ABT-Anterior Basal Ganglia/Thalamus; PBT- Posterior Basal Ganglia/Thalamus
**Neuropsychological Correlates of WMH**

All participants underwent the full neuropsychological battery. Exploratory Pearson correlational analyses were conducted in order to investigate relationships between total DWH, total PVH and total lacune and the neuropsychological test scores. The only variables significantly associated were total volume of lacune with the Rey complex figure ($r = -0.35, P = 0.009$) and the Benton Line Orientation ($r = -0.28, P =$...
This relationship was further investigated by correlating the lacunar volumes by region and the two neuropsychological tests. The Rey-Osterrieth complex figure was negatively correlated to the lacunar volumes in the occipital ($r = -0.28, P = 0.04$) and medial middle frontal ($r = -0.38, P = 0.004$) regions. The Benton Line Orientation task showed a significant negative relationship with the lacunar volumes in the posterior temporal ($r = -0.26, P = 0.05$) and inferior parietal regions ($r = -0.30, P = 0.02$).

**DISCUSSION**

This study presents descriptive data on the patterns of WMH and their possible correlates in a sample of mild to moderate AD patients. We used semi-automatic methods to segment three brain tissue types (GM, WM, and CSF) and quantify WMH volume and location (PVH, DWH) in 13 lobar regions. We also separately measured volume and location of lacunar infarcts within the brain.

The strength of the current study lies in its rigorous brain imaging protocol that involved a robust T1-weighted segmentation protocol, combined with a brain regional parcellation technique and a reliable semi-automated lesion segmentation protocol which allowed for sensitive measurement of WMH in this sample of patients. This study is one of the first to use quantitative volumetry of WMH in an AD sample with varying severities of white matter disease and to differentiate objectively between different WMH subtypes, including DWH, PVH and lacunar infarcts within the WMH. Another important feature is the description of lacunar infarcts within the WMH. This data allowed for a systematic cross-sectional description of the topography of co-occurring CVD in AD patients.

Firstly, our results are consistent with previous studies in healthy elderly and AD patients suggesting a relationship between CVD risk factors and larger volumes of WMH.
(Yoshita et al., 2006; Capizzano et al., 2004; DeCarli et al., 2005) Overall, individuals with a greater score on the CVD risk factor scale showed higher volumes of WMH, both PVH and DWH. In contrast, no relationship was found between total volume of lacunar infarcts and CVD risk factors. CVD risk factors such as hypertension are common in the elderly population and have been shown to be associated with increased WMH volumes in normal elderly controls (Longstreth, Jr. et al., 1996; Longstreth, Jr. et al., 2005) as well as increased brain atrophy (Firbank et al., 2007). It is possible that decreased brain perfusion in individuals with CVD risk factors such as hypertension contributes to the increased PVH, which represent a watershed zone supplied by the long penetrating pial arteries. The basal ganglia and thalamus are likewise end-zones for arterioles arising from the basal arteries. Alternatively, damage to the venous system may also contribute to WMH as a function of venous insufficiency that may increase with aging due to venous collagenosis and cerebrovascular risk factors (Gao et al., 2007). Whether the relationship between CVD risk factors and WMH is specific to PVH or also includes DWH may depend on the way these hyperintensities are classified. One previous study suggested a stronger relationship than found in this study between hypertension and DWH; however, that study used a different method of classifying PVH and DWH, with DWH being defined as any hyperintensity greater than 1cm from the border of the ventricles (Firbank et al., 2007). In the current study, PVH included any hyperintensity that connected to the ventricle in three-dimensional space, thereby including those at larger distances than 1cm from the border of the ventricle.

Using a semi-automatic method of quantification, the topography of DWH and PVH was similar, with both WMH subtypes found most commonly in the frontal and
parietal brain regions. Specifically WMH were most common in the middle frontal and inferior parietal regions, accounting for approximately 60% of total hyperintensity volume. These regions correspond to the anterior and posterior horns of the lateral ventricles and the centrum semiovale. Using our method of distinguishing between PVH and DWH, only 9% of total WMH were classified as DWH, which showed a similar regional distribution as the PVH. These similar topographical findings may support previous research suggesting that the categorical distinctions between PVH and DWH may be arbitrary (DeCarli et al., 2004) with both types of hyperintensity showing similar lobar patterns of distribution. Although there have been numerous studies examining the topography of WMH, the findings are inconclusive. The discrepant findings may be due to a variety of differing instruments used for measurement, such as visual rating scales (de Groot et al., 2000), semi-automatic and automatic volumetric techniques (Admiraal-Behloul et al., 2005; DeCarli et al., 2005), and also arbitrary methods of assigning hyperintensities as either periventricular or deep white. This study confirms that PVH are ubiquitous in elderly patients with dementia as previously reported (Wen et al., 2004), and perhaps more so in AD patients as every patient in our sample had PVH and ninety-five percent had DWH. In general, the topography of WMH in AD seemed to maintain a similar pattern to that seen in normal aging (Wen et al., 2004; DeCarli et al., 2005), which suggests that it may be a manifestation of brain aging independent of AD. Yet, recent research suggests that microbleeds in both normal aging and AD, thought to be associated with amyloid angiopathy, may be also related to increased presence of WMH (Pettersen et al., 2008).

Unlike most other studies of WMH, this study not only explicitly measured
lacunar infarcts both as a ratio of TRV and as a percent of total WMH, but also distinguished between the WMH sub-types that the lacune was found within. By measuring the actual number of lacunar infarcts and generating information on the both the average size of distinct lesions and the total brain lacune volumes, different patterns within DWH and PVH were investigated. The subcortical grey matter region (basal ganglia/thalamus) had the largest ratio of lacunar infarct per total hyperintensity (4.7%), which is not surprising given that lacunar infarcts are frequently seen in the basal cerebral artery end zone. An additional finding from this analysis was that lacunes found within the PVH were larger in overall volume than those found within DWH. Yet, on average, a greater number of lacunes were found within DWH. Interestingly, higher ratios of lacune relative to WMH volume in a region were most often found in regions associated with smaller volumes of WMH, for example, the occipital and anterior temporal regions. This was in contrast to the frontal regions which had a larger total volume of lacunes but a relatively smaller ratio given the total WMH in that area.

Although WMH and lacunar infarcts have been suggested to be two distinct expressions of small vessel disease, the patterns seen in the current study suggest that the differences may be more complex. One possibility is that the different patterns reported (on imaging, clinical and brain-behavior correlational studies) between non-necroctic WMH and lacunes may be linked to differences between the fluid-filled infarcts or lacunes found within the PVH and those found within the DWH. The topography of the fluid-filled infarcts (lacunes) found within the periventricular region is not the same as those lacunes found withing the DWH. Periventricular lacunes showed a topography that was very similar to that seen within the non-necrotic PVH, with a smaller number of
discrete lacunes (maximum number of lacunes in PVH was 6) but a larger overall volume. This is different than the patterns of lacunes found within the deep-white region which were much greater in number but smaller in volume. It is possible that previous studies, which utilized visual rating scales without including or measuring lacunar infarcts, may not have properly distinguished between lacunes and non-necrotic WMH, thereby combining the lacunes with both PVH and DWH into one, as opposed to two or three separate pathologies. This lack of differentiation may have contributed to some studies reporting large differences in the patterns, distribution and correlates of DWH and PVH, when in actuality the variability between PVH and DWH was a result of the inclusion of lacunes. This might explain the different opinions on whether these two are different even though they share commonalities, such as similar risk factors and pathogenesis (Sachdev et al., 2005).

Another possible explanation for the differences between lacunes found within the DWH and those found within the PVH lies in the relationship between DWH and lacunes. The larger number of discrete lacunes found within the DWH may imply that DWH are an early stage of infarction or “incomplete infarction” which may or may not progress to complete cystic necrosis as measured by CSF intensity. We were unable to test this hypothesis in the current study, but further research focusing on the progression patterns of all three lesion types would help to clarify this.

The only significant neuropsychological correlates in the current study were the association of total volume of lacunes with both the Rey-Osterrieth complex figure and the Benton Judgment of Line Orientation. Previous research has suggested that lacunes and WMH are independently associated with cognition; however, that study relied on
more general measures of overall cognition (van der Flier et al., 2005). In the current study, cognitive tests included specific tasks probing a variety of cognitive domains. Interestingly, neither PVH nor DWH were related to performance on the Dementia Rating Scale, a task that assesses general cognition. It is possible that in this sample the presence of a co-occurring Alzheimer’s pathology prevented any independent cognitive deficits caused by the presence of WMH to show statistical significance. Alternatively, the non-necrotic WMH may not be as damaging to brain connectivity as lacunar infarcts and therefore may be less disruptive to cognitive performance.

When results were further examined based on location or region of lacunar infarct we found modest correlations in regions not only associated with the tasks, but also associated with greatest volumes of lacunes relative to total WMH. Interestingly, both tasks in which significant relationships were found assess visuospatial perception and organization. The Benton Judgment of Line Orientation has been associated with lesions in posterior brain regions and in the current study, lacunes in the posterior temporal regions were correlated with decreased performance on this task. Similarly, the Rey-Osterrieth Complex figure performance is associated with both frontal and right parieto-temporal lesions (Lezak, Muriel, Howieson, & Loring, 2004), and we did find a relationship between decreased performance and large volumes of frontal lacunes. In addition, occipital lobe lacunes modestly correlated with performance on the Rey-Osterrieth complex figure. One component of the Rey-Osterrieth involves visual perceptual function (not only organization but the ability to perceive the stimulus) which is typically associated with parieto-occipital deficits; the relationship between decreased performance and occipital lacunes may reflect this circuitry. In the current study, few
relationships between cognition and subcortical ischemic vascular disease were discerned, likely due to the limited sample size and the large number of neuropsychological tasks included in the analysis. Furthermore, most patients were mildly to moderately impaired, with limited range on some of the more sensitive tasks, such as memory.

There are several limitations in the present research that should be acknowledged. Firstly, the number of patients should be increased in future studies to allow for a broader distribution of WMH severity and lacunes and to better explore brain behavior relationships. Although the findings were comparable to a number of large population studies, larger data sets that segment the different hyperintensities are important in both elderly controls and AD populations. Also the data in this study are cross-sectional and correlational, so any inferences as to causality cannot be made, nor was lesion progression determined. Longitudinal data examining the rate of progression of WMH subtypes in different regions may help to establish whether these subtypes are distinct pathologies, by focusing on regional progression patterns and relationships between rate of progression and other AD markers. In addition, by following patients longitudinally, differences in a larger selection of neuropsychological tests may be more perceptible because AD may progress at different rates. The current patients were all at a similar, mild to moderate stage of the disease, and therefore the variability within the neuropsychological tests may have been too limited to see correlations.

This is to our knowledge one of the first studies to examine WMH in a group of AD patients using a quantified method of brain segmentation that distinguished between PVH, DWH and lacunar infarcts in different brain regions. Patients with AD seem to
have similar patterns of WMH as seen in larger samples of normal elderly, with both PVH and DWH found most prominently in the frontal and parietal brain regions. Lacunar infarcts showed different patterns than WMH, with the majority found in the basal ganglia/thalamus regions and were correlated to decreased performance on visuospatial tasks.
REFERENCE LIST


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Ref Type: Abstract


Ramirez, J. L. N. & B. S. E. (6-3-2005). Lesion Explorer: an MRI segmentation processing technique for 3D volumetric analyses of lacunes, periventricular,
and deep white matter hyperintensities.

Ref Type: Unpublished Work


INTRODUCTION

Medial temporal lobe (MTL) atrophy is a commonly used magnetic resonance imaging (MRI) marker for Alzheimer’s disease (AD) atrophy. Pathological studies have suggested that the MTL is an early-targeted site of the primary neuropathological cascade in AD, with volume loss occurring in the early stages of AD. MRI white matter hyperintensities (WMH) are also a common finding in patients with AD with between 95 to 100% of AD patients showing some degree of WMH on MRI (Levy-Cooperman, Ramirez, Lobaugh, & Black, 2008). A relatively understudied area in the field of WMH research involves the relationship between WMH and brain atrophy measures in AD patients. WMH are thought to be a symptom of microvascular pathology. Recent autopsy studies have suggested that vascular pathology, in the form of cortical or lacunar infarcts, may accelerate the clinical expression of dementia in AD (Snowdon et al., 1997). A relationship between the severity of WMH and MTL atrophy could provide further evidence to suggest an enabling role for vascular pathology in the pathogenesis of AD.

One previous study reported a relationship between subcortical hyperintensities and medial temporal atrophy (van der Flier et al., 2004), where the combination of MTL atrophy and hyperintensity volume distinguished AD patients from normal elderly controls. Another study, however, did not find a relationship between subcortical hyperintensities and MTL atrophy; rather they found that hyperintensities were related only to decreased total cortical gray matter volumes (Du et al., 2005). Although some
studies have begun to examine the relationship between WMH and MTL atrophy, the results are not conclusive; nor have they specifically examined relationships between the different subtypes of WMH and MTL atrophy. Some research suggests that deep white hyperintensities (DWH) are more strongly associated with ischemic damage, and that periventricular hyperintensities (PVH) (especially those with smooth delineated borders) result from demyelination and subependymal gliosis of nonischemic origin (Spilt et al., 2006). Therefore, demonstrating a relationship between DWH and MTL atrophy could more strongly support a relationship between ischemic vascular pathology and AD.

The majority of WMH research to date has focused on the relationship between WMH and cognition, and brain function and atrophy. However, recent cross-sectional studies have begun to investigate differences in WMH patterns between men and women, with a number of population-based studies reporting a higher prevalence of WMH among women than men (DeCarli et al., 2005; Longstreth, Jr. et al., 1996; de Leeuw et al., 2001). Furthermore, a longitudinal study reported that women had a greater progression of DWH than men, but saw no differences in PVH, suggesting that sex may influence the pathogenesis of DWH with different clinical consequences for men and women (van den Heuvel et al., 2004).

The aim of the current study was to investigate the relationship between WMH and a validated measure of medial temporal lobe atrophy, namely its narrowest width (Gao et al., 2003; Gao et al., 2004), in a group of mild to moderate AD patients with varying severity of WMH. This study also sought to investigate possible sex differences in severity and distribution of WMH in AD patients.
METHODS

Participants

Participants were volunteers of the Sunnybrook Dementia Study, recruited from the Cognitive Neurology Clinic at Sunnybrook Health Sciences Centre, a University of Toronto academic healthcare institution. All patients received a comprehensive clinical evaluation, including detailed medical history, neurological examination, routine laboratory investigation and neuropsychological testing with a standardized test battery. Individuals were excluded if the MRI and neuropsychological testing were separated by more than 12 weeks, or if the MRI scans were technically inadequate for volumetric analysis. All subjects were greater than 60 years of age and had to be fluent in English to be included. Secondary causes of dementia (other than subcortical vascular disease), concomitant neurological or psychiatric illnesses, or significant co-morbid systemic illness were exclusionary. This protocol was reviewed and approved by the institutional Research Ethics Board, and written informed consent was obtained from all subjects or their substitute decision makers.

In the current study, patients had the historical profile typical of AD with insidious onset of short term memory loss and gradual decline of function and cognition. 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 et al., 1984), and Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (American Psychologioical Association, 1994) criteria for dementia. Diagnosis was reached by consensus of two clinicians based on these criteria, including one year of observation. Demographic information was collected on all participants including age at time of MR scan, number of years of
education, sex, and duration of cognitive impairment and presence of cerebrovascular risk factors. In this sample, the diagnosis of “Possible” AD was based only on the presence of sufficient subcortical cerebrovascular disease (CVD), to contribute to dementia, but patients with extensive SH did not meet criteria for Vascular Dementia (VaD); that is, patients were excluded if they met the National Institute for Neurological Disorders and Strokes (NINDS) - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) criteria for possible or probable VaD, which requires focal neurological signs in addition to imaging evidence of CVD. Patients diagnosed with possible AD due to other causes or focal memory deficit alone were not included. Presence of silent cortical strokes on MRI was also exclusionary.

**MR Image Acquisition**
Magnetic resonance images were acquired on a 1.5 Tesla Signa scanner (GE Medical Systems, Milwaukee, Wisconsin) and complied with the consensus panel imaging recommendations on Vascular Cognitive Impairment (Hachinski et al., 2006b). Three image sets were acquired in the same imaging session: T1-weighted (an axial 3D SPGR with 5ms TE, 35ms TR, 1 NEX, 35° flip angle, 22 x 16.5 cm FOV, 0.859 x 0.859 mm in-plane resolution, and 1.2 to 1.4mm slice thickness depending on head size), PD and T2-weighted (interleaved axial dual-echo spin echo with TEs of 30 and 80 ms, 3s TR, 0.5 NEX, 20 x 20cm FOV, 0.781 x 0.781mm in-plane resolution, and 3mm slice thickness).

**MR Image Post-processing**
Brain extraction and automated tissue segmentation was accomplished using a modified version of previously described methods (Kovacevic et al., 2002). Proton density (PD) and T2-weighted images were coregistered to the T1-weighted image using
automated image registration software (AIR, v.5.2 23). The PD/T2 images were used to extract brain and subdural cerebrospinal fluid (CSF) and used to mask the T1. The masked T1 was segmented using a T1-based segmentation which is a robust and reliable tissue segmentation protocol optimized for elderly and AD populations (Phantom: coefficient of agreement=0.97, Scan-Rescan differences: Global < 1% of total intracranial volume (TIV), Local < 0.15% of TIV) (Kovacevic et al., 2002).

Brain region parcellation was accomplished using a modified version of our previously described methods for semi-automated brain region extraction (SABRE) (Dade et al., 2004). SABRE is a highly reliable method which parcellates each individual brain into 26 brain regions proportional to individual head sizes (Inter-class Correlation range: 0.97-0.99 for individual tissue classes in each region). A set of easily identified landmarks were traced on the masked T1 images using the 3D rendering and region of interest (ROI) modules in the ANALYZE software package (Biomedical Imaging Resource, Mayo foundation, Rochester, MN, USA): the central sulcus, sylvian fissure, parieto-occipital sulcus, anterior commissure and posterior commissure. An in-house program combined these landmarks with the Talairach proportional grid system (Talairach J, 1988) to generate individualized maps of 13 lobular regions in each hemisphere.

White matter hyperintensity segmentation was accomplished using a previously described method called Lesion Explorer © (Quddus, 2004; Ramirez, 2005; Ramirez J, 2007) . This semi-automated procedure used an intensity cutoff based on a weighting of the PD and T2 images to define putative WMH. A 3-dimensional voxel dilation procedure was applied to the T1-segmentation's ventricular volumes using a 2 voxel
dilation factor in ANALYZE. This was to account for partial volume effects and for the elimination of "pencil thin" periventricular hyperintensities which are believed to reflect T2-weighted flow artifacts from CSF. The output was then manually edited by a trained operator who accepted relevant hyperintensities (based on concurrent evaluation of PD and T2 weighted images) to generate final lesion volumes (ICC range for 26 SABRE brain regions: 0.96-0.99). Lobar volumes of different tissue types were normalized to the supratentorial TIV and expressed as a percentage. When examining WMH distribution by brain region, WMH volumes were expressed as mean percent of the three tissue types contributing to a specific region of interest (GM + WM + CSF- ventricular and sulcal= Total Regional Volume), this was referred to as Total Regional Volume (TRV). PVH were identified as those hyperintensities that connected to the ventricles in three-dimensional space. DWH in the current study only included those hyperintensities that were discrete and not connected to the ventricles, including those in the deep nuclei.

**Medial Temporal Width Acquisition**

We used the thinnest width of the MTL as a focal measure and total CSF (ventricular and sulcal CSF) volume as an index of generalized atrophy to investigate the relationship between WMH and atrophy. MTL width was measured using a previously published protocol having good reliability, with ability to distinguish patients from normal controls (Gao et al., 2003; Gao et al., 2004). The thinnest MTL width of each hemisphere was measured between the anterior-posterior boundaries of the midbrain, at the level of the inter-collicular sulcus, on MR images oriented along the long axis of the hippocampus. Realignement and reformatting of the MR images were conducted using ANALYZE AVW™ Software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) on a Sun workstation (Sun Microsystems, Mountain View, CA).
Statistical Analysis

Data analysis was performed using SPSS software version 12.0 (SPSS Inc, Chicago, Ill). Differences between men and women on demographic and clinical variables were assessed using independent samples t-tests for continuous variables. Sex differences for all volumetric measures were examined using multivariate analysis of variance (MANOVA). Spearman correlation analyses were used to investigate relationships between WMH volumes and measures of MTL atrophy as well as global brain volumes. Multiple stepwise linear regression models were used to investigate relationships between WMH (PVH and DWH) and atrophy measures (MTL width, volume of CSF) with age entered as a covariate. Correlations between the independent variables were investigated to check for collinearity. The level of significance was set at p<0.05.

RESULTS

Participants

Refer to Chapter III-A.

WMH and Measures of Focal Brain Atrophy

Overall WMH volume was negatively correlated with MTL width (r= -0.38, p =0.003). Correlations were also investigated between PVH, DWH and total lacune volume. DWH and PVH were strongly correlated (r=0.75, p<0.001) and total lacune volume was significantly correlated with both PVH (r=0.8, p<0.001) and DWH (r=0.72, p<0.001). As a result, subtypes were entered separately into regression analyses due to concern for collinearity. MTL width served as the dependent variable, with the WMH subtypes entered as independent variables and age entered as a covariate. Volume of
PVH ($r^2=0.17\ p=0.006$) emerged as significantly correlated with MTL volume, accounting for 12% of the variance in the model (with age accounting for < 5%). Figure 1 depicts the relationship between MTL width and PVH volume. As expected, total WM volumes were negatively correlated to total WMH ($r = -0.56, p <0.001$), with both PVH ($r = -0.56, p <0.001$) and DWH ($r = -0.24\ p =0.03$) showing negative relationships to WM volume. In contrast, GM volumes, and CSF volumes did not show any relationship to total WMH volume.

![Graph](image)

**Figure 1: Relationship between volumes of periventricular hyperintensities and medial temporal lobe atrophy.**

**Sex Differences in Global and Regional Tissue Compartment Volumes**

MANOVA results indicated a significant overall group difference for mean brain volumes ($F_{(7, 51)}=6.4, p < 0.001$). Univariate analyses showed that TIV for women was
approximately 162cm³ smaller than that for men (1295.3 ± 92.6cm³ for men versus 1132.9 ± 108.1cm³, F (1, 57) = 36.2, P =0.001). Differences in head size were accounted for by dividing all total brain volumes (GM, WM, CSF, PVH, and DWH) by the TIV. When examining patterns in relation to specific ROIs, WMH volumes were expressed as a percentage of a particular SABRE region and referred to as TRV. Hemispheric differences were also investigated, and because no significant differences were found between left and right hemisphere for GM, WM, CSF, PVH, DWH, and total WMH in men and women, the left and right volumes were combined.

Mean sex differences for total tissue type are summarized in Table 1. Statistically significant sex differences existed for CGM (F (1, 57) = 9.54, P=0.003) and DWH (F (1, 57) = 4.78, P=0.03), with women showing larger relative volumes of GM and DWH. Mean volumes of GM in 13 ROIs are shown in Figure 2. Additional MANOVA investigating sex differences in GM and DWH were conducted to determine differences in volume when divided by brain region. Women showed larger GM volumes in the following regions: middle frontal (F(1,57)= 6.27, P=0.02); inferior frontal (F(1,57)= 9.35, P=0.003); medial inferior frontal (F(1,57)= 7.50, P=0.008); occipital (F(1,57)= 11.88, P=0.001); anterior temporal (F(1,57)= 7.05, P=0.01); posterior temporal (F(1,57)= 8.12, P=0.02); posterior basal ganglia/thalamus (F(1,57)= 4.57, P=0.04) and the medial superior frontal region (F(1,57)= 4.14, P=0.047. Sex differences in DWH were examined regionally. Women had significantly larger DWH in the superior (F(1,57) = 5.34, P=0.02) and middle frontal regions (F(1,57) = 4.12, P=0.047). Trends toward higher DWH volumes were also seen in the inferior parietal (F(1,57) = 3.3, P=0.07) and medial superior frontal regions (F(1,57) = 2.9, P=0.09).
Table 1. Demographic findings and Total brain MRI segmentation findings (N=59)

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<th>Measure</th>
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<th>Female (n=35)</th>
<th>t/test</th>
<th>P-value</th>
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<td>76.1 (6.7)</td>
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<td>Years of Education</td>
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<td>12.9 (3.5)</td>
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<td>CVD Risk Factor (%)</td>
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<td>16.5 (16.2)</td>
<td>12.6 (13.4)</td>
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<td>0.33</td>
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<td>CGM*</td>
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<td>41.3 (2.2)</td>
<td>43.2 (2.4)</td>
<td>9.54</td>
<td>0.003</td>
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<td>WM*</td>
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<td>sCSF*</td>
<td>21.9 (3.5)</td>
<td>23.1 (3.7)</td>
<td>21.1 (3.2)</td>
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<td>vCSF*</td>
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Data presented as mean (SD).

* MRI volume measures are expressed as % mean (SD) of TIV.

**Lacunar Infarct volumes are expressed as % mean (SD) of total brain WMH.

WM= white matter; CGM=cortical grey matter; vCSF= Ventricular Cerebrospinal fluid; sCSF= Sulcal Cerebrospinal Fluid; DWMH= deep white matter hyperintensities; PVWMH= periventricular white matter hyperintensities.
Figure 2: Regional Grey Matter Volumes for Men and Women. Mean ROI grey matter volumes for men and women. Consistent differences were seen between men and women in grey matter volumes for eight of thirteen regions. * = p <0.05; ** = p <0.01
SF- Superior Frontal; MF- Middle Frontal; IF- Inferior Frontal; MIF- Medial Inferior Frontal; MSF- Medial Superior Frontal; MMF- Medial Middle Frontal; SP- Superior Parietal; IP-Inferior Parietal; O-Occipital; AT-Anterior Temporal; PT- Posterior Temporal; ABT-Anterior Basal Ganglia/Thalamus; PBT- Posterior Basal Ganglia/Thalamus
Figure 3: Mean regional DWH Volumes for Men and Women. Women had a greater amount of DWH in the superior and middle frontal regions. Trends toward DWH volume differences were also seen in the medial superior frontal and the inferior parietal regions. * = p < 0.05; § = p < 0.1

SF- Superior Frontal; MF- Middle Frontal; IF- Inferior Frontal; MIF- Medial Inferior Frontal; MSF- Medial Superior Frontal; MMF- Medial Middle Frontal; SP- Superior Parietal; IP-Inferior Parietal; O-Occipital; AT-Anterior Temporal; PT- Posterior Temporal; ABT-Anterior Basal Ganglia/Thalamus; PBT- Posterior Basal Ganglia/Thalamus

DISCUSSION

This study investigated the relationship between WMH and medial temporal lobe width, and sex differences in volumes of WMH in a group of AD patients.

Current findings demonstrated a relationship between WMH volumes and MTL width which was related to PVH volume but not DWH. One possible explanation for this finding is that at least some cases of PVH may be indirectly related to the amyloid

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angiopathy associated with Alzheimer’s pathophysiology, while DWH may be directly related to arteriolar disease. Amyloid angiopathy is thought to underlie the increased presence of microbleeds in AD compared to elderly controls (Cordonnier, 2006). One recent study reported a relationship between occipital microbleeds and PVH (Pettersen, JA et al, 2008). If PVH are more common in AD related to amyloid angiopathy, then they might be expected to correlate with MTL width, which has been shown to reliably decreased in AD (Gao et al., 2003; Gao et al., 2004). No significant relationship was found between the WMH subtypes and CSF volumes. Research on the relationship between WMH and whole brain atrophy (measured by total brain CSF volumes or total brain parenchyma) has yielded mixed findings, including a report of an association between whole brain atrophy and WMH volume (Firbank et al., 2007) and a report showing no difference in brain atrophy between patients with and without WMH (de Leeuw, Barkhof, & Scheltens, 2004a).

A few studies have previously examined the relationship between WMH and brain atrophy measures specifically in AD patients. One study reported an interaction between subcortical hyperintensities and medial temporal atrophy (van der Flier et al., 2004), where the combination of MTL atrophy and large hyperintensities distinguished AD patients from normal elderly controls. Another study did not find a relationship between subcortical hyperintensities and MTL atrophy (Du et al., 2005); rather they found that hyperintensities were related only to decreased cortical gray matter volumes. Similarly, Capizzano et al. also reported an inverse relationship between WMH volumes and total brain GM volumes (2004). Another study that focused on the relationship between brain atrophy and lacunes reported dramatic hippocampal and
cortical grey matter atrophy in dementia patients with large volumes of WMH and lacunes (Fein et al., 2000). Our study is among the first to examine the relationship between atrophy and WMH, categorized by PVH, WMH and lacunes. While both PVH and DWH can be caused by cerebral ischemia, different vascular mechanisms may be involved (Wen, Sachdev, Chen, & Anstey, 2005). One possible explanation is that a threshold effect exists, whereby larger lesion volume (as in PVH), implies a more widespread ischemic injury that impacts on the hippocampus.

Sex differences in this group of AD patients were consistent with previous studies of normal aging (de Leeuw et al., 2001; Wen et al., 2004; Longstreth, Jr. et al., 1996) suggesting only small differences between men and women in total WMH volumes. The differences found in the current study were similar to those reported in a large population based study (N= 2200) which reported similar brain volumes of 1343.2 ± 109.2 cm³ for men and 1182.5 ± 104.4 cm³ for women. However, unlike the current study, which found larger GM volumes in women in a number of regions, the previous study reported larger frontal lobe grey matter and CSF volumes in men (DeCarli et al., 2005). Upon further examination of WMH, the current study revealed larger DWH in women, specifically in the frontal lobes. Larger volumes of WMH in women have previously been attributed to a marked decrease of estrogen concentrations in post-menopausal women (de Leeuw et al., 2001). Estradiol exerts both neurotrophic and neuroprotective actions against neurodegenerative diseases and brain injury (Wise, Dubal, Wilson, Rau, & Liu, 2001). Estrogen has also been associated with beneficial effects on cerebrovascular disease through neuronal repair mechanisms (Wise et al., 2001). These hormonal effects may be related to the sex differences in whole brain and WMH
volumes. Further research, which focuses on the relationships between hormones, AD and WMH are warranted. Interestingly, our findings were of a similar magnitude as those seen in the larger studies of normal aging (small differences between men and women), suggesting that a concurrent diagnosis of AD may not increase or decrease the sex differences seen in studies of white matter disease as one might expect (de Leeuw et al., 2001; DeCarli et al., 2005; Wen et al., 2004). Thus the incidental white matter disease seen in AD (as opposed to infarcts) may be an aging change that is superadded to the AD process.

The findings from the current study suggest that different mechanisms may be involved in the etiology of DWH and PVH. Sex differences in WMH volumes suggest the possibility of different clinical consequences in women with AD, while the relationship between PVH and MTL atrophy illustrates the complexity of AD pathology and the need for further quantitative studies in both AD and normal elderly populations.
REFERENCE LIST


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MRI scans. Journal of Neurological Sciences 226[1-2], 148-149.

Ref Type: Abstract

Ramirez, J. L. N. & B. S. E. (6-3-2005). Lesion Explorer: an MRI segmentation processing technique for 3D volumetric analyses of lacunes, periventricular, and deep white matter hyperintensities.

Ref Type: Unpublished Work


CHAPTER IV
PROGRESSION OF WHITE MATTER HYPERINTENSITIES IN ALZHEIMER’S DISEASE

INTRODUCTION

White matter hyperintensities (WMH) are a common finding on T2-weighted or proton density (PD) magnetic resonance imaging (MRI) in older adults and have been studied extensively over the past two decades. Visual rating scales and more recently volumetric measures have been used to quantify and study the anatomical distribution of these hyperintensities; however, relatively little is known about the regional rate and patterns of progression of these hyperintensities. Some research suggests that WMH may progress gradually over time as vascular risk factors increase, eventually resulting in extensive subcortical white matter disease which can cause cognitive decline and gait disturbance (Pugh et al., 2002; Schmidt et al., 2003b).

The few studies that have examined longitudinal WMH changes have yielded conflicting results. In large population studies, progression has usually been measured using visual rating scales (Whitman, Tang, Lin, & Baloh, 2001; Schmidt, Fazekas, Kapeller, Schmidt, & Hartung, 1999; de Leeuw et al., 2005; Longstreth, Jr. et al., 2005). For example, the Austrian Stroke Prevention Study investigated progression of WMH at three year follow-up in a sample of normal elderly and found that approximately 17% of the sample showed progression of WMH (Schmidt et al., 1999; Schmidt et al., 2003b). Another large study reported progression in 28% of a sample of community-dwelling elderly, with the majority of those showing minimal progression. Unlike the Austrian study, they also found a relationship between progression and cognitive decline (Longstreth, Jr. et al., 2005). Although visual rating scales are more efficient and
convenient for large multi-centre studies, they are less well-suited for measuring change in white matter hyperintensity severity (Prins et al., 2004).

Few studies have focused on the measurement of changes in WMH volumes over time using volumetric techniques, which may be more accurate and precise, specifically when focusing on possible increases or decreases in WMH volumes. Volumetric analyses have quantified changes in volume using measures of total WMH. One study in particular found no difference in the rate of WMH progression across a number of disease groups (dementia with Lewy Bodies, Parkinson disease dementia and Alzheimer’s Disease (AD)) and healthy elderly controls (Burton et al., 2006). A few studies have distinguished between progression in periventricular (PVH) and deep white hyperintensity (DWH), with one study suggesting that only confluent lesions progress (Schmidt et al., 2003a), and another reporting that the rate of progression was greater in DWH (Sachdev et al., 2007). Although the findings from the small number of studies investigating change in WMH volumes vary within the literature, the finding that baseline amount of WMH is the strongest predictor of increased amount at follow-up is consistent (Burton et al., 2006; Sachdev et al., 2007; de Leeuw et al., 2005; Schmidt et al., 2003a; Schmidt et al., 2003a). Previous studies have not specifically investigated increases in regional lacunar infarct volume. Lacunes often appear as focal WMH on T2-weighted or PD-MRI and many visual rating scales and quantitative analyses do not take these “black holes” into account since T1-weighted scans are not simultaneously examined. Lacunes are not always discerned on Fluid Attenuated Inversion Recovery (FLAIR) imaging, now included in standard clinical acquisitions, as they can sometimes be missed because of the thick slices routinely used. Lacunes have been previously
associated with subtle cognitive dysfunction and decreased frontal lobe metabolism (Reed et al., 2001; Reed et al., 2004), yet very little is known about how or if they progress over time. Similarly, the relationship between progression of WMH and cognitive decline is not yet understood.

The purpose of the current study was to investigate patterns of progression, over approximately one-year follow-up, in a group of mild to moderate AD patients using a semi-automated lesion segmentation program. The aim was to quantitatively measure a) progression in total WMH volume, DWH and PVH volumes, b) lacunar infarct progression patterns c) progression patterns of WMH in 26 lobar regions and d) to examine the relationship between WMH progression and change in performance on cognitive testing.

METHODS

Participants

Participants were volunteers of the Sunnybrook Dementia Study, recruited from the Cognitive Neurology Clinic at Sunnybrook Health Sciences Centre, a University of Toronto academic healthcare institution. All patients received a comprehensive clinical evaluation, including detailed medical history, neurological examination, routine laboratory investigation and neuropsychological testing with a standardized test battery. Inclusion and exclusion criteria of this study have been described elsewhere (Levy-Cooperman et al., 2007a). Briefly, for this study, men and women greater than 60 years of age were included from the larger study if they had the historical profile typical of AD, usually with an insidious onset of short term memory loss and gradual decline of function and cognition. All patients met the National Institute of Neurological and
Communicative Disorders and Stroke – Alzheimer’s Disease and Related Disorders

Association criteria for probable or possible AD (McKhann et al., 1984). Demographic information was collected on all participants including age at time of MR scan, number of years of education, sex, and duration of cognitive impairment and presence of cerebrovascular risk factors. The “Possible AD” diagnosis was based on the presence of sufficient subcortical cerebrovascular disease, to contribute to dementia, but patients with extensive WMH did not meet the current criteria for Vascular Dementia (VaD) (Erkinjuntti, 1994). Presence of “silent” cortical strokes on MRI also excluded the patient. Sixty-four patients were enrolled in this study. From these original 64, 5 were excluded due to technical problems with MRI. At follow-up, 28 patients had dropped out, leaving a total of 31 subjects (53%). Patients were lost to follow-up due to death (n=2) or inability to undergo neuropsychological or MRI testing due to disease progression (n=23) and three MRI scans were not useable due to motion. Characteristics of the participants lost to follow-up were further investigated. Compared to the follow-up participants, those who dropped out were older (F= 6.6, P= 0.01), had greater PVH at baseline (F =4.8, P= 0.03), greater medial temporal lobe atrophy (F= 4.6, P= 0.04), performed worse on the baseline Mini-Mental Status Examination (MMSE) (F= 5.3, P= 0.02) and Dementia Rating Scale (DRS) (F= 10.1, P= 0.002), and were more likely to have cerebrovascular disease (CVD) risk factors ($\chi^2 = 3.9$, P=0.04).

Cerebrovascular Risk Factors

Vascular risk factor profile was systematically assessed from the subject’s history and a review of pertinent medical records to create a baseline vascular risk factors composite score ranging from 0-10. The Vascular Risk factors scale assigned scores for
treated (2 pts) or untreated hypertension (4 pts), treated (1 pt) or untreated hyperlipidemia (2 pts), diabetes (2.5 pts), and currently (1.5 pts) or quit smoking (0.5 pts). Information on history of stroke, TIA, myocardial infarction, angina, and peripheral vascular disease and an extensive medication list (anticonvulsants, antidepressants, antipsychotic, antithrombotics, antihypertensive, ACE inhibitors, beta-blockers, cognitive enhancers, diabetes treatments, hormone replacement therapy, lipid lowering medication, NSAIDS, thyroid treatment) was also collected.

**Neurobehavioral Assessment**

Cognitive function was assessed with a battery of neuropsychological tests selected for use with this study population administered at baseline and at end of follow-up. Specific tests were chosen with the intention of: a) providing an assessment of general cognitive function and b) to index impairments in specific cognitive domains known to associated with particular brain regions, c) The tests included the following:

- **Digit Span**: *Forward Digit Span and Backward Digit Span* are tests of attention and working memory, where the number of digits repeated correctly is noted. They measure the efficiency of attention (freedom from distractibility) and mental tracking (how many bits of information a person can attend to at once and repeat in reverse order), respectively.

- **Wisconsin Card Sorting Test** – 64 item version (WCST), (Heaton, 1981) measures abstracting ability and set-shifting capacity. Key measures used in this study were the number of perseverative responses (perseverative errors- a measure of mental flexibility) and the number of categories achieved (a measure of abstraction and set-shifting).

- **Trails Making Test Part A & B**- is a test that probes visuomotor tracking, divided attention and cognitive flexibility. Trails-A requires an individual to draw lines
sequentially connecting 25 encircled numbers distributed on a sheet of paper. Task requirements are similar for Trails-B except the person must alternate between numbers and letters in serial order (e.g., 1, A, 2, B, 3, C, etc.). The score on each part represents the amount of time required to complete the task. The Trails B task tests the ability to shift attention during an ongoing activity and to allocate attention to more than one type of stimulus at a time (Spreen et al., 1991).

- **Phonemic Fluency (FAS):** tests verbal fluency and the ability to initiate and sustain responses. In this task as many words as possible beginning with the letters F, A, or S had to be generated within one minute. Participants were instructed not to repeat the same words, use pronouns, or derivatives of the same words.

- **Semantic Fluency (SF):** tests semantic fluency and the ability to initiate and sustain responses. In this task as many words as possible in the category animals had to be generated within 60s. Participants were instructed not to repeat the same words.

- **Dementia Rating Scale** (Mattis, 1976): the overall score indexes global cognitive functioning emphasizing executive function and consists of subsets for attention, initiation and perseveration, conceptualization, construction and verbal and non-verbal short term memory.

- **Benton Line Orientation** (Benton A.L. et al., 1983): tests visuospatial orientation and attention. This task requires the identification of lines matching a target with an array of differently oriented lines.

- **Rey Osterrieth Complex Figure –Copy** (Osterrieth, 1944): tests perceptual organization, visual/spatial perception and construction. Patients are asked to copy a complex geometric stimulus on a blank sheet of paper.
- California Verbal Learning Test- Acquisition Sub-Score (Delis D.C. et al., 1987) –

tests verbal learning and memory. Patients are required to learn semantically related
words from a 16-item list over a series of 4 trials.

**MR Image Acquisition**

All brain imaging was obtained at Sunnybrook Health Sciences centre on a 1.5 T
Signa scanner (GE Medical systems) and complied with the consensus panel imaging
recommendations on Vascular Cognitive Impairment (Hachinski et al., 2006a). Three
image sets were acquired in the same imaging session: T1-weighted (an axial 3D SPGR
with 5ms TE, 35ms TR, 1 NEX, 35° flip angle, 22 x 16.5 cm FOV, 0.859 x 0.859 mm in-
plane resolution, and 1.2 to 1.4mm slice thickness depending on head size), PD and a
T2-weighted (interleaved axial dual-echo spin echo with TEs of 30 and 80 ms, 3s TR,
0.5 NEX, 20 x 20cm FOV, 0.781 x 0.781mm in-plane resolution, and 3mm slice
thickness).

**MR Image Post-processing**

Brain extraction and automated tissue segmentation was accomplished using a
modified version of previously described methods (Kovacevic et al., 2002). This is a
hierarchical stepwise approach utilizing information from multiple MR images - the
PD/T2 images for brain extraction, followed by a T1-based segmentation where local
intensity histograms are fitted to four Gaussian curves to derive cut-offs used to classify
each voxel as white matter (WM), grey matter (GM), or cerebrospinal fluid (CSF). It is
a robust tissue segmentation protocol optimized for elderly and Alzheimer Disease
populations (Phantom Data: coefficient of total agreement=0.97, Scan-Rescan maximal
differences in normal and elderly AD patients: Global < 1% of Total Intracranial Volume
(TIV) in all tissue classes, Local < 0.15% of TIV). The original procedure was modified
so that segmentation was performed in T1-acquisition space, in an attempt to avoid interpolation errors resulting from image re-slicing.

Brain region parcellation was accomplished using a modified version of our previously described methods for semi-automated brain region extraction (SABRE) (Dade et al., 2004). SABRE is a highly reliable method which parcellates each individual brain into 26 brain regions proportional to individual head sizes (Inter-class Correlation range: 0.97-0.99 for individual tissue classes in each region). A set of easily identified landmarks were traced on the masked T1 images using the 3D rendering and region of interest (ROI) modules in the ANALYZE software package (Biomedical Imaging Resource, Mayo foundation, Rochester, MN, USA): the central sulcus, sylvian fissure, parieto-occipital sulcus, anterior commissure and posterior commissure. An in-house program combined these landmarks with the Talairach proportional grid system (Talairach J, 1988) to generate individualized maps of 13 lobular regions in each hemisphere. Figure 1 shows a map of the 13 regions which consisted of: superior, middle, and inferior frontal, superior, middle, and inferior medial frontal, superior and inferior parietal, anterior and posterior temporal, anterior and posterior basal ganglia and thalamus, and occipital regions.
White matter hyperintensity segmentation was accomplished using previously described methods (Quddus et al., 2004; Ramirez, 2005; Ramirez, 2007). This is a semi-automated procedure with an automated component which uses an intensity cut-off based on a weighting of the PD and T2 images. The SH segmentation output is then manually edited to generate lesion volumes (Inter-class Correlation for 26 SABRE brain regions: 0.96-0.99). Lobar volumes of different tissue types were normalized to total intracranial volume (TIV) and expressed as a percentage. When examining WMH distribution by brain region, WMH volumes were expressed as mean percent of the three tissue types contributing to the specific region of interest (GM + WM + CSF- ventricular and sulcal= Total Regional Volume), this is referred to as Total Regional Volume (TRV). PVH were identified as those hyperintensities that connected to the ventricles in three-dimensional space. DWH in the current study only included those hyperintensities that were discrete and not connected to the ventricles, including...
those in the deep nuclei. Medial Temporal Width (MTL) width was obtained using a previously published protocol (Gao et al., 2003; Gao et al., 2004). The thinnest medial temporal lobe width of each hemisphere was measured between the anterior-posterior boundaries of the midbrain at the level of the inter-collicular sulcus, on an image–oriented scan orientated along the long axis of the hippocampus.

**Lacune Measurements**

Using the T1 segmentation, an additional subcategory of WMH containing fluid-filled cysts, less than 2.0 cm\(^3\) was included as lacunar volumes. Lacune volumes were reported as a ratio of total WMH in that region. Lacunes were distinguished from Virchow-Robin spaces during the manual hyperintensity selection using the T1-image for confirmation. Virchow-Robin spaces were defined as isointense to CSF on T1, T2, and PD sequences (Barkhof, 2004).

**Statistical Analysis**

Data analysis was performed using Statistical Package of Social Sciences software version 12.0 (SPSS Inc, Chicago, Ill). Summary statistics of demographic information and WMH measurements at baseline and follow-up are reported as mean (range). All total WMH volumes were divided by TIV to correct for differences in brain size between subjects. Change in WMH was also expressed as a percentage of the baseline TIV. Normally distributed (Total WMH progression, PVH progression) data was assessed using paired student’s t-test. Nonparametric statistical tests (Wilcoxon signed rank test) were used to test non-normally distributed data (DWH progression, Kolmogorov Smirnov Z=1.6, P=0.01 and Lacune progression, Kolmogorov Smirnov Z=1.4, P=0.03). We calculated difference scores at each subject’s follow-up visit (follow-up minus baseline) for each cognitive test. Linear regression models were then
used to investigate relationships between WMH (PVH and DWH) and change on cognitive testing. Correlations between the independent variables were investigated to check for collinearity. The level of significance was p<0.05.

RESULTS

Subject Characteristics

The demographic and clinical characteristics of the subjects with and without follow-up are summarized in Table 1. The mean ± SD age of the subjects was 74.5 ± 5.0 years and 45% were men. Follow-up time was no less than one year, with a mean of 1.5±0.7 years.

Table 1. Baseline demographic details for subjects with and without follow-up data.

<table>
<thead>
<tr>
<th></th>
<th>With follow-up (n = 31)</th>
<th>Without follow-up (n=28)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>74.2±5.0</td>
<td>78.3±6.9</td>
<td>F= 6.58, p&lt;0.05 *</td>
</tr>
<tr>
<td><strong>Sex (men)</strong></td>
<td>14 (45%)</td>
<td>10 (36%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
<td>14.4±3.6</td>
<td>12.5±3.7</td>
<td>ns</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>25.2±2.8</td>
<td>23.0±4.3</td>
<td>F= 5.28, p&lt; 0.05 *</td>
</tr>
<tr>
<td><strong>DRStot</strong></td>
<td>123.9±10.4</td>
<td>114.4±12.4</td>
<td>F= 10.1, p&lt;0.01</td>
</tr>
<tr>
<td><strong>Hypertension§</strong></td>
<td>11 (35%)</td>
<td>8 (32%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Hyperlipidemia§</strong></td>
<td>5 (16%)</td>
<td>5 (20%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>1 (3%)</td>
<td>4 (16%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>CVD Risk Factors</strong></td>
<td>13 (42%)</td>
<td>19 (68%)</td>
<td>$\chi^2$=3.98, p&lt;0.05 *</td>
</tr>
<tr>
<td><strong>MTL Width (mm)</strong></td>
<td>11.3±3.6</td>
<td>8.17±4.5</td>
<td>F=4.62, p&lt;0.05 *</td>
</tr>
</tbody>
</table>

* P <0.05
§ data missing on 4 subjects
Baseline WMH (excluding lacunes)

Baseline WMH data are presented in Table 2. The majority of baseline WMH were found in the frontal and parietal regions, specifically in the middle frontal and inferior parietal region, and accounted for 60% of total WMH volumes. PVH and DWH were examined separately and accounted for 91% and 9% of total hyperintensity volume, respectively. General topography for PVH and DWH was similar, with the majority of lesion volumes deriving from the inferior parietal, middle and medial frontal, and occipital regions. Fifty-eight percent (18/31) of the patients had PVH that exceeded 0.5% of TIV, which is considered to be a clinically relevant threshold given that it has been associated with decreased frontal metabolism, greater CVD risk factors and decreased performance on executive tasks (DeCarli et al., 1995; Boone et al., 1992).

Progression of WMH

Forty-five percent of AD patients showed an increase in total WMH volume at follow up (mean (cc) 0.67; 3.3% change), but this increase was not statistically significant (t = -1.07, p = 0.29). When DWH and PVH progression were examined separately, total DWH volumes showed a significant increase at follow-up (mean (cc) 0.37; 26% change; Wilcoxon signed ranks; Z = -2.9, P = 0.004). No significant progression was seen in PVH at one year follow up (Mean (cc) 0.3; t = -0.7, p = 0.49). Mean WMH, DWH and PVH at baseline and follow-up are presented in Figure 2. Rate of progression was calculated by dividing the change in volume from baseline to follow-up by the total number of months. Annual rates of progression were as follows: overall WMH- increase of 0.02 cm³/year; PVH-a decrease of 0.02 cm³/year; DWH –increase of 0.16 cm³/year. Additional analyses investigated 26 brain regions to determine which regions showed change from baseline to follow-up. These findings are presented in
Figure 3 for DWH and Figure 4 for PVH. DWH showed the greatest volume increases, in the left superior frontal \((Z=-2.8, p<0.01)\), both right and left middle frontal (Left: \(Z=-2.4, p<0.05\), Right: \(Z=-3.9, p<0.001\)), and right inferior frontal regions (\(Z=-2.3, p<0.05\)).
Table 2. Longitudinal White Matter Hyperintensity Volumes

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Followup</th>
<th>Volume Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WMH (cm$^3$)</td>
<td>13.4 (0.2- 58)</td>
<td>14.1 (0.04- 52.5)</td>
<td>0.67 (-5.8 - 10.7)</td>
</tr>
<tr>
<td>Total WMH (%TIV)</td>
<td>1.1 (0.02- 5.2)</td>
<td>1.2 (0.01- 4.7)</td>
<td>0.06 (-0.5- 0.8)</td>
</tr>
<tr>
<td>Total DWH (cm$^3$)</td>
<td>1.4 (0- 12.6)</td>
<td>1.8 (0.0- 13.6)</td>
<td>0.37 (-2.1- 5.2)</td>
</tr>
<tr>
<td>Total DWH (% TIV)</td>
<td>0.1 (0- 0.9)</td>
<td>0.15 (0- 1.0) *</td>
<td>0.03 (-0.19 -0.39)</td>
</tr>
<tr>
<td>Total PVH (cm$^3$)</td>
<td>11.9 (0.2- 52.2)</td>
<td>12.2 (0.04- 48.5)</td>
<td>0.30 (-3.7- 5.2)</td>
</tr>
<tr>
<td>Total PVH (% TIV)</td>
<td>1.0 (0.01- 4.7)</td>
<td>1.0 (0.01- 4.4)</td>
<td>0.01 (-0.34 -0.61)</td>
</tr>
<tr>
<td>Total Lacune (cm$^3$)</td>
<td>0.11 (0- 0.9)</td>
<td>0.14 (0.0- 0.9)</td>
<td>0.03 (-0.2- 0.37)</td>
</tr>
<tr>
<td>Total Lacune (% total WMH)</td>
<td>0.85 (0- 5.9)</td>
<td>0.94 (0- 3.6)</td>
<td>0.89 (0.0- 4.1)</td>
</tr>
</tbody>
</table>

%TIV - percent intracranial volume.
WMH: white matter hyperintensities; PVH: periventricular hyperintensities
DWH: deep white matter hyperintensities
* p < 0.05
**Figure 2:** Total brain, periventricular, and deep white hyperintensity at baseline and one-year follow-up. Volumes corrected for head size using total intracranial volume (TIV). Deep white hyperintensities showed a significant increase at one-year follow-up (mean (cc) 0.37; 26% change; Wilcoxon signed ranks; $Z = -2.9$, $P = 0.004$).
**Figure 3:** Mean DWH volumes of AD patients at baseline and follow-up scan time for 26 regions of interest (ROI). Volumes corrected for ROI size using total regional volume (TRV). Greatest volume increases were seen in the left superior frontal, right and left middle frontal, and right inferior frontal regions. SF- Superior Frontal; LF- Middle Frontal; IF- Inferior Frontal; MIF- Medial Inferior Frontal; SP- Superior Parietal; IP- Inferior Parietal; O- Occipital; AT- Anterior Temporal; PT- Posterior Temporal; AB- Anterior Basal Ganglia/Thalamus; PB- Posterior Basal Ganglia/Thalamus; MSF-Medial Superior Frontal; MMF- Medial Middle Frontal. * = p <0.05; ** = p<0.001
Figure 4: Mean PVH volumes of AD patients at baseline and follow-up scan time for 26 regions of interest (ROI). Volumes corrected for ROI size using total regional volume (TRV). SF- Superior Frontal; LF- Middle Frontal; IF- Inferior Frontal; MIF- Medial Inferior Frontal; SP- Superior Parietal; IP- Inferior Parietal; O- Occipital; AT- Anterior Temporal; PT- Posterior Temporal; AB- Anterior Basal Ganglia/Thalamus; PB- Posterior Basal Ganglia/Thalamus; MSF- Medial Superior Frontal; MMF- Medial Middle Frontal.
Baseline Lacune Measures

At baseline, lacunar infarcts accounted for 0.85% of total WMH volume (see Table 2). Eighty-six percent (n=51) of the original sample (N=59) had at least one lacunar infarct. Of these, 71% (n=42) had at least one lacune in the deep white matter, and 69% (n=41) had at least one lacune within the periventricular white matter. In this group, the mean number of total brain lacunar infarcts was 5.5 (range 0-22), with a mean of 4 (range 0-20) lacunar infarcts found within the DWH, and a mean of 1.5 (range 0-6) lacunes within the PVH. Lacunar infarct volumes were separately measured as a subcategory within the original WMH segmentation. These volumes were reported as percent of total WMH volume and were separated according to tissue type and location. The highest ratio of lacunar infarct to regional WMH volume was found in the posterior basal ganglia and thalamus region accounting for 4.7% of that region’s total WMH volume, but only 1.4% of whole brain lacune volume. The occipital and anterior temporal regions also had a larger ratio of lacune to WMH with lacunes accounting for 3.2% and 3.4% of regional WMH volume, respectively. These two regions combined accounted for 11.7% of the total brain lacunar volume. Of those patients who had one year follow up (n=31), 87% had at least one lacune at baseline.

Follow-up Lacune Measures

At follow-up lacunar infarcts accounted for 0.94% of total WMH volume. Eighty-seven percent (n= 27 of 31) of the follow-up sample had at least one lacunar infarct; 65% (n=20 of 31) had at least one lacune in the deep white matter; and 77% (n=24 of 31) had at least one lacune within the periventricular white matter. The average change in discrete lacune counts is presented in Figure 5. Overall lacune counts did not significantly increase between time one and time two (Z= -0.8, P = 0.42). Only
periventricular lacunes showed a significant increase in the number of discrete lesions between time one and time two (Mean Time 1 = 1.32 (1.24), Mean Time 2 = 2.19 (1.55); t=3.7, p=0.001). The average size of periventricular lacunes increased from baseline (Mean lacune volume = 0.24cc) to follow-up, suggesting that both the number of discrete lacunes and the average size of discrete lacunes was increasing (Mean lacune volume = 0.46cc; Mean Change 0.02).

Figure 5: Discrete lacune counts (total, periventricular and deep white) at baseline and one year follow-up.

Lacune volume progression was also measured as a change in the ratio of lacune volume per total WMH. At follow-up, total lacune volume did not significantly increase (Mean (cc) 0.03; 27% change; Z= -1.3, P = 0.18). Lacunes within the deep white matter and the periventricular white matter were examined separately and neither showed a significant
increase in volume from time one to time two; however periventricular lacunes did show an increase in volume, albeit not statistically significant, which is consistent with the increase in number and average size of discrete lacunes reported (DW- Mean change (cc) 0.005; 19% change; Z=-.08, p=0.9; PV- Mean change (cc) 0.026; 30% change; Z= -1.5, P=0.12). When progression of lacune volume was investigated by brain region, significant increases were only found in the medial inferior frontal region (Mean change (cc) 0.004; 328% change; Wilcoxon signed ranks; Z= -2.7, p<0.01). Lacune volumes at baseline and follow-up are presented in Figure 6.

**Figure 6:** Mean lacune volumes of AD patients at baseline and follow-up scan time for 13 regions of interest (ROI). Volumes corrected for ROI size using total regional volume (TRV). SF- Superior Frontal; LF- Middle Frontal; IF- Inferior Frontal; MIF- Medial Inferior Frontal; SP- Superior Parietal; IP- Inferior Parietal; O- Occipital; AT- Anterior Temporal; PT- Posterior Temporal; AB- Anterior Basal Ganglia/Thalamus; PB-
Neuropsychological Measures

To determine whether progression in WMH volume was associated with rate of cognitive decline, we investigated the relationship between change in scores on neuropsychological tests and change in WMH volume from baseline to follow-up (Table 3). Significant decline in cognitive performance was seen from baseline to follow-up on the following tests: MMSE, DRS-total score and DRS-Attention, DRS-Initiation, DRS-Memory, Semantic Fluency, Rey Copy, Trails A and Trails B (See Table 3).

**Table 3** Cognitive test results at baseline and follow-up.

<table>
<thead>
<tr>
<th>Test</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini Mental State Examination</td>
<td>25.7 (2.76)</td>
<td>23.3 (4.4)</td>
<td>t=3.6</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Dementia Rating Scale</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>125.8 (9.3)</td>
<td>118.6 (14.2)</td>
<td>t=3.5</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Attention</td>
<td>35.0 (1.9)</td>
<td>33.7 (3.1)</td>
<td>t=2.3</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Initiation</td>
<td>31.6 (4.0)</td>
<td>29.4 (5.9)</td>
<td>t=2.5</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Construction</td>
<td>5.41 (0.9)</td>
<td>5.1 (1.3)</td>
<td>t=1.2</td>
<td>P=0.24</td>
</tr>
<tr>
<td>Conceptualization</td>
<td>35.3 (3.4)</td>
<td>34.6 (3.5)</td>
<td>t=0.9</td>
<td>P=0.36</td>
</tr>
<tr>
<td>Memory</td>
<td>18.4 (3.7)</td>
<td>15.9 (4.4)</td>
<td>t=4.1</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Phonemic Fluency</td>
<td>32.5 (12.2)</td>
<td>30.3 (12.6)</td>
<td>t=1.5</td>
<td>P=0.14</td>
</tr>
<tr>
<td>Semantic Fluency</td>
<td>12.1 (4.3)</td>
<td>10.7 (4.4)</td>
<td>t=3.1</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Wisconsin Card-Sorting Task</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Categories</td>
<td>2.4 (1.5)</td>
<td>2.0 (1.4)</td>
<td>t=1.2</td>
<td>P=0.23</td>
</tr>
<tr>
<td>No. of perseverations</td>
<td>11.3 (8.2)</td>
<td>10.8 (7.1)</td>
<td>t=0.2</td>
<td>P=0.81</td>
</tr>
<tr>
<td>Trail Making Test A</td>
<td>64.4 (48.1)</td>
<td>83.5 (79.4)</td>
<td>t=-2.1</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Trail Making Test B</td>
<td>151.0 (63.0)</td>
<td>206.5 (128.5)</td>
<td>t=2.5</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Forward Digit Span</td>
<td>8.6 (2.0)</td>
<td>8.1 (1.7)</td>
<td>t=1.7</td>
<td>P=0.09</td>
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<tr>
<td>Backward Digit Span</td>
<td>6.0 (2.7)</td>
<td>5.9 (2.3)</td>
<td>t=0.5</td>
<td>P=0.60</td>
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<td>Benton Judgement of Line Orientation</td>
<td>19.3 (9.1)</td>
<td>20.2 (9.1)</td>
<td>t=-1.4</td>
<td>P=0.17</td>
</tr>
<tr>
<td>Rey Osterrieth Complex Figure</td>
<td>27.5 (8.3)</td>
<td>25.1 (8.4)</td>
<td>t=2.4</td>
<td>P&lt;0.05</td>
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<tr>
<td>California Verbal Learning Test</td>
<td>25.0 (9.9)</td>
<td>21.2 (9.8)</td>
<td>t=2.0</td>
<td>P=0.05</td>
</tr>
</tbody>
</table>

Data are presented as means (SD). P value from paired t test.

Progression in total WMH was associated with decline in the semantic fluency task (r=0.37, P = 0.04). When DWH and PVH were investigated separately, only DWH
progression correlated with decline in semantic fluency \((r=0.42, \ P = 0.02)\). Progression of PVH was correlated with decreased scores on the DRS-initiation subsection \((r=0.39, \ P = 0.04)\). We also performed two linear regression analyses with percent change of PVH, DWH and lacunes entered as the independent variables, adjusted for age and years of education, with scores on semantic fluency and DRS-initiation as the dependent variables. DWH progression was the only significant predictor of decline in semantic fluency, accounting for 9% of the variance \((R^2= 0.53, \ P=0.04)\), in addition to age and years of education (which accounted for 44% of the variance). Decline in the DRS-initiation subcategory was not significantly predicted by any WMH measures. Progression of lacunar volumes did not correlate with cognitive decline on any neuropsychological tests in this AD sample.

**DISCUSSION**

In this study, we investigated patterns of WMH and lacune progression in a group of 31 probable/possible AD patients with repeat MRI scans at an approximate interval of one year. Overall WMH volume increased in just under half of the AD patients at one year follow-up. These findings are relatively consistent with some previous studies in healthy elderly; however, very few of the studies have looked specifically at short-term progression in AD. Many of the studies have used visual rating scales relying on qualitative data and therefore, could not report on actual volume of hyperintensity progression (de Leeuw et al., 2005; Longstreth, Jr. et al., 2005; Whitman et al., 2001; Schmidt et al., 2003b). Visual rating scale studies reported progression in approximately 20-30% of patients over two to three years, while the few volumetric studies report a range of 20-90% of patients who show progression at two- to three-year follow-up. Other
volumetric studies also report higher total WMH volume progression than was found in the current study, ranging between 30-40% total volume increases. However, most of those studies had a longer follow-up time, reporting at both three and six years of follow-up (Schmidt et al., 2003a; Schmidt et al., 1999; Sachdev et al., 2007). We are aware of only one other study that examined progression in AD at one year follow-up, which reported similar findings of 0.08% increase in WMH volume (as a percent of brain volume) (Burton et al., 2006). Further evaluation of the current sample at longer follow-up times would be necessary to establish whether findings of long term progression between centers using different volumetric techniques are comparable.

Although the majority of WMH were found in the periventricular region, in this sample, only DWH showed a significant increase after one year. This finding is consistent with one study which also reported significant increases in deep white matter, but this study reported an overall larger volume of DWH throughout the brain (Sachdev et al., 2007). It is likely that the methodology used to distinguish between PVH and DWH was different from the technique used within our sample (Sachdev et al., 2007). One other study examined sex-differences in progression of WMH, and reported DWH progressed at a much faster rate in women than men (van den Heuvel et al., 2004). However, the study also reported a greater progression of DWH compared to PVH in both sexes. Unfortunately, most studies distinguish between PVH and DWH differently. In the current study, WMH were considered “periventricular” if connected to the ventricle at any point. Thus, DWH were mostly discrete, focal hyperintensities. It is unclear whether the pathophysiology of PVH and DWH differ; in fact, the debate on whether a distinction should be made between these two hyperintensities is ongoing.
Current opinions vary, with some suggesting that categorical distinctions are arbitrary due to the high correlation between PVH and DWH (DeCarli et al., 2004; Swartz, 2002), while others suggest that the two are different even though they share commonalities, such as similar risk factors and pathogenesis (Sachdev et al., 2005). The differences in progression reported to date tend to support the latter concept. It should be noted that one study suggested that the confluent hyperintensities (generally associated with PVH) were malignant and discrete lesions benign, but that study did not distinguish between dilated perivascular spaces and lacunes. The current study used a semiautomatic technique for volumetric measurement of WMH and lacunes, and extra care was taken in determining which discrete lesions were accepted, using a detailed decision-making protocol, to exclude dilated perivascular spaces. It is possible that previous studies included both types of pathology, which may have inflated lacune volumes. A consensus on how to distinguish between PVH, DWH, lacunes and Virchow-Robin spaces would be desirable so that studies from different centres could be more accurately compared.

As far as we are aware, the current study was the first to investigate regional progression of WMH in AD. Across the 26 lobar regions, DWH progression was greatest in frontal brain regions, specifically the superior, middle and inferior frontal regions. This finding may explain some of the functional disturbances that have been reported in individuals with WMH. Decline in executive functioning and decreased performance on tasks of attention, speed and information processing often reported in individuals with WMH may be related to frontal lobe susceptibility to cerebrovascular disease (DeCarli, 2003; Tullberg et al., 2004; Pugh et al., 2002; Kuo & Lipsitz, 2004). In fact, we found a relationship between progression of both DWH and total WMH and decline in
performance on a semantic fluency task. Fluency tests have been suggested to reflect predominantly language and frontal lobe function and are sensitive to AD early in the disease process. Generally, patients with early AD show changes in the medial temporal lobe, and the frontal lobes may not be affected by the disease until later stages. This finding may be a result of the progression of AD pathology, but, it is possible that the executive dysfunction that is common in AD may in part relate to susceptibility of frontal-mediated tests to WMH.

Increases in PVH volume were also associated with decline on cognitive testing. Progression of PVH correlated with decreased performance on the initiation/perseveration subsection of the dementia rating scale. This subsection specifically tests verbal fluency, mental control and resistance to interference and has been shown to be useful in discriminating between controls and patients with small vessel disease (Wong, Mok, Tang, Lam, & Wong, 2007). Similar findings were reported in the PROSPER study (van den Heuvel, 2006), which used a similar measurement to distinguish between PVH and DWH and found that non-demented elderly showed a relationship between PVH and decline in information processing tasks. Previous studies have reported that AD patients show a greater baseline level of WMH when compared to normal controls (Burton et al., 2006), and most studies have shown that amount of baseline WMH predicts severity of progression (Sachdev et al., 2007; de Leeuw et al., 2005). It is possible that in some AD patients, WMH progression is more severe (de Leeuw et al., 2005), specifically in frontal regions and therefore is related to greater decline in executive function. If this is the case, it underlines that in AD patients with WMH and decreased performance on tasks of executive function, management should
emphasize treatment of vascular risk factors, such as hypertension, metabolic syndrome etc (de Leeuw et al., 2004; Park et al., 2007), in an attempt to lessen cognitive decline.

This study was among the first to measure the progression of lacunes in an AD sample. Total brain lacune volume increased in 52% of patients at follow-up. A count of discrete lacunes at baseline and follow-up showed a significant increase in the number of discrete periventricular lacunes. The average size of periventricular lacunes also increased at follow-up. These findings suggest that not only are increases in lacunes at one year follow-up due to the emergence of new lacunes, but it is likely that existing lacunes may also be growing in size. Whole brain periventricular lacune volume showed a slight increase in volume, but this finding did not reach statistical significance in the current sample. Additional analyses with a larger sample may be warranted. Although, the basal ganglia region had the largest volume of lacunes (relative to total WMH), the greatest increase in ratio of lacune to WMH was found in the medial inferior frontal brain region. Our findings did not support a relationship between progression of lacunes and decline in cognition, which is in keeping with previous studies (Mungas et al., 2005). The finding of large volumes of lacunes in the basal ganglia was not surprising given that the term lacune is often associated with lesions to the basal ganglia or internal capsule (Halsey, 1986); however, what was novel was the significant increase found in the medial frontal region. Within the current study, the frontal lobe seemed to be at risk for increases in WMH at follow-up. Other follow up studies in normal elderly at longer intervals have also reported greater progression in frontal areas (Sachdev et al., 2007). Further studies examining lacune progression patterns in non-demented elderly as well
as AD samples, will help clarify whether these frontal regions are at greater risk in AD patients.

The progression of WMH has been assessed in previous studies; however, many have used visual rating scales which are subject to observer bias. The current study used a semi-automatic quantitative technique to measure progression of hyperintensities in a number of brain regions, after correction for TIV. It is interesting that not all our patients WMH progressed at one year follow-up, and we did see reductions in WMH in some subjects. This reduction has been reported in previous studies (Burton et al., 2006; Sachdev et al., 2007), yet it is still unclear whether this reduction is a measurement artifact or an actual pathological change. Interestingly, PVH were more likely to decline in volume at one year follow-up compared to DWH, which prompted the investigation of ventricular size at baseline and follow-up as a possible explanation for this decline. Ventricular volume did show a significant increase from baseline to follow-up (Baseline vCSF Volume= 50 cm\(^3\), Follow-up vCSF Volume= 57 cm\(^3\); \(t= 9.27, p < 0.001\) suggesting that the decline in PVH volume may be due to the ventricles growing larger.

This study was also among the first to measure the progression of lacunes using a quantitative method, and to measure the volume of lacunar infarction within the WMH in each region and the whole brain. This allowed the measurement of lacunes which are obscured when using visual rating scales, because they are hidden within regions that have large volumes of discrete or confluent WMH. Some of the limitations of the current study include the small sample size associated with a high drop-out rate, which could lead to survival bias. In general, our follow up sample were younger and had fewer CVD risk factors. Patients who had the most severe WMH and CVD risk factors
appeared less able to withstand the cognitive testing and possibly the follow-up MRI scan. As a result, our follow-up sample may have had less severe WMH than is typical for probable AD patients.

In contrast to previous studies in healthy aging populations, our findings suggest that progression of WMH in AD may be more influenced by the type or location of lesion rather than total lesion volume. Further studies should focus on the volumetric measurement of lacune progression in varying disease groups as well as healthy elderly controls in order to establish what regions may be especially susceptible to these infarcts.
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Swartz, R. H. (2002). Evaluating the impact of cerebrovascular disease on cognition using quantitative MRI.

Ref Type: Unpublished Work


One of the major challenges, for both research scientists and clinicians in the fields of aging, dementia and stroke, is to better characterize and understand, both at the basic science and translational levels, the distinctions and interactions between Alzheimer’s disease (AD) and Cerebrovascular Disease (CVD). On neuropsychological testing, AD is typically characterized by memory deficits while CVD manifests itself by focal and/or executive function deficits. Imaging and autopsy studies of AD patients classically show brain atrophy, thought to start in the medial temporal region (Braak et al., 1995), while similar studies of patients with subcortical CVD characteristically show large and small-vessel infarcts and WMH (Babikian & Ropper, 1987). On cognitive testing, subcortical CVD can affect information processing, speed of response and other executive functions (Pugh et al., 2002). Although the etiologies of the two diseases are distinct, they often co-occur and due to the heterogeneity of AD, can be difficult to differentiate. In fact, recent studies have proposed that the distinction between neurodegenerative and cerebrovascular disease may not be valid since the overlap between the two conditions is so commonly observed (van der Flier et al., 2007).

Coexisting CVD and AD is not a new discovery. Their co-occurrence was reported as early as 1907, when Dr. Alois Alzheimer identified the first patient with what came to be known as Alzheimer’s Disease, a 51-year-old woman, with behavioral and cognitive disturbance. Upon pathological examination, this patient exhibited amyloid plaques and neurofibrillary tangles, now known to be pathological markers of the disease; however, she also exhibited atherosclerotic changes (O'Brien, 2006; Graeber, Kosel, Grasbon-Frodl, Moller, & Mehraein, 1998; Kalaria, 2002). Pathologically ”pure”
AD and “pure” Vascular Dementia (VaD) have proven to be relatively less frequent than combined disease in population autopsy series reporting the spectrum of neurodegenerative and cerebrovascular diseases (Snowdon et al., 1997; Anonymous, 2001; Lim et al., 1999). For this reason, current research is focusing less on distinguishing between the two pathologies, and more on viewing both disorders along a continuum with VaD at one end and AD at the other, with various degrees of mixed pathology occurring in between (van der Flier et al., 2007). The reported prevalence rates of VaD, AD and mixed disease vary. Autopsy series report mean rates of VaD at 4-10% (range 0 to 85%) and rates of mixed disease ranging from 2 to 56%, depending on community, hospital or specialty clinic referral base (Lim et al., 1999; Snowdon et al., 1997; Anonymous, 2001; Kalaria, 2002; DeCarli, 2004). Autopsy studies also suggest that in AD patients, the risk of cerebrovascular lesions is much higher than in other degenerative diseases, with a range of 18 to 80% of AD brains showing additional CVD pathology (Jellinger et al., 2007; Nolan, Lino, Seligmann, & Blass, 1998; Nagy et al., 1997). Multiple autopsy and large population studies have reported that virtually all late-onset AD patients exhibit vascular changes, and have also reported a significant relationship between higher Braak stages and the prevalence of CVD (Jellinger & Attems, 2005). These findings, although somewhat contradictory depending on what definitions are used to define vascular changes, suggest that mixed disease is likely the most common pathological substrate of dementia amongst elderly patients with dementia (Anonymous, 2001).

A difficulty that has arisen within this field, and one that may have influenced the sometimes conflicting findings to date, is the lack of a common terminology for the
continuum of vascular changes in dementia. Both probable AD and probable VaD are relatively well defined by the National Institute of Neurological and Communicative Disorders and Stroke –Alzheimer’s Disease and Related Disorders Association criteria (NINCDS-ADRDA) and National Institute for Neurological Disorders and Strokes (NINDS) - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) criteria, respectively. Defining the different degrees of mixed disease has proved more difficult. Recently, Vascular Cognitive Impairment was introduced as an umbrella term to encompass Vascular Dementia, Vascular Cognitive Impairment No Dementia, and mixed vascular and neurodegenerative conditions such as AD in which cerebrovascular disease is a contributing factor (Skoog, 2004). However, Vascular Cognitive Impairment is also used to describe the milder potentially prodromal stage of VaD (Hachinski & Bowler, 1993; Hachinski, 1994), similar to amnestic Mild Cognitive Impairment as a precursor to AD (Peterson et al 1999). Lack of commonly agreed definitions poses obstacles in this field of research. Another terminological minefield is the description of WMH, which can range from cystic lesions in the white matter to subcortical hyperintensities embracing white matter and deep nuclei lesions or periventricular and deep white hyperintensities, the latter including white matter or deep nuclei hyperintensities that are away from the ventricles. Different techniques for measuring these hyperintensities lead to conflicting interpretations of what qualifies a lesion as periventricular or deep white. Furthermore, if these “bright spots” are aptly named WMH, should the subcortical nuclei not be measured and included when generating hyperintensity volumes?
Controversies in this field are not just terminological. The classification of these diseases and their influence on one another are other areas that have yet to be understood. Both additive and synergistic effects of mixed disease have been proposed, suggesting on one hand that the two diseases may represent independent co-occurring pathologies, and on the other, that small vessel and large vessel disease can exacerbate the pathological cascade of AD and in turn, that AD through amyloid angiopathy can cause ischemia, as well as micro and macro hemorrhages (Chen et al., 2006). Data suggests that individuals with similar degrees of overall cognitive impairment can differ on the extent of underlying AD pathology, especially if there is also vascular pathology involved (Anonymous, 2001; van der Flier et al., 2007; Snowdon et al., 1997). Specifically, the co-occurrence of injury from vascular disease can worsen the cognitive impairment at earlier stages of AD (Esiri et al., 1999). It is important to note, however, that many of the studies that have suggested this interplay between the two diseases included only patients with actual lesions or infarcts (Snowdon et al., 1997) as opposed to strictly so-called “incidental WMH”, which may not even be reported in autopsy reports of AD. Studies that have examined WMH as seen on CT or magnetic resonance imaging (MRI) specifically, have been inconsistent, some reporting little to no influence of WMH, and others reporting that a certain threshold volume must be met before any influence of WMH is seen on cognition (Boone et al., 1992; DeCarli et al., 1995).

Since relatively little is understood about the particular contribution of WMH to the heterogeneity of AD, and because both the topography of WMH and their progression patterns have not been previously established, the current study set out to describe relationships between white matter disease and AD. Specifically, this project
focused on patients with mild to moderate AD, who had varying severities of WMH in order to address the following objectives: 1) to establish whether measures of global WMH severity correlated with brain perfusion patterns on single photon emission computed tomography (SPECT) 2) to quantify volumes and report on the topography of WMH and lacunar infarcts 3) to determine the correlations between WMH and cognition 4) to track progression patterns of WMH and lacunes over one year 5) to ascertain the relevance of location of WMH and lacunes to decline in cognitive function.

The first study investigated the relationship between severity of WMH, using a visual rating scale, and frontal lobe perfusion in AD patients. This study did not find a relationship between the presence of WMH and frontal perfusion in an AD population. This contrasts to some quantitative studies, which have reported a relationship between frontal lobe function and the volume of subcortical ischemic vascular disease (SIVD) in a dementia population selected based on the presence of lacunes (Tullberg et al., 2004). These authors reported small differences in executive function between participants with greater WMH severity and those with minor or no WMH, in keeping with some previous research suggesting a relationship between SIVD and dysexecutive syndrome in the elderly population (Esiri et al., 1999; DeCarli et al., 1995).

The next step was to evaluate the severity of WMH in the same sample using a quantitative volumetric analysis. A new methodology was used (Lesion Explorer), which was designed not only to measure total WMH volumes, but also to differentiate between periventricular hyperintensities (PVH), deep white hyperintensities (DWH) and lacunar infarcts within 26 brain regions. This analysis provided the most comprehensive descriptions to date of WMH topography in an AD sample. The value of measuring
WMH subtypes separately was shown in this analysis, as we were able to determine that the brain regions affected most prominently by WMH were similar for both PVH and DWH. In both cases, the majority of involvement was found in the frontal and parietal brain regions. These findings lend some support to the notion that PVH and DWH may represent similar pathologies at different stages. This study was also one of the first to examine topographical patterns of lacunar infarcts in AD, and suggested a possible relationship between DWH and lacunes as they were found more often within these lesions.

In Chapter 3b, sex differences in brain tissue volumes were investigated (as the ability to measure total supratentorial intracranial volume allowed for head size correction and meaningful comparison of men and women). This analysis showed that men and women with AD do differ in brain volume, with women showing less atrophy than men of similar age and AD severity. On the other hand, women tended to have larger volumes of WMH, with the greatest differences found in the frontal regions. A relationship between medial temporal lobe atrophy and WMH was also confirmed by the current results, which suggested a negative relationship between the severity of hyperintensities, specifically PVH, and the extent of medial temporal lobe (MTL) atrophy.

The next step in the investigation of WMH topography was to establish if, and where, WMH progress in AD patients and if so, in what regions. Specifically, if WMH progress over time, are certain brain regions associated with greater increases in WMH pathology and does change correlate with progressive loss of cognitive abilities? Some earlier studies had suggested a relationship between cognitive impairment and a
threshold amount of WMH in AD (Boone et al., 1992). Therefore, it was hypothesized that increased volumes of WMH would correlate with decreased performance on neuropsychological tests. Since the regions most affected involved the frontal lobes, executive function tasks were hypothesized to be most influenced by progression of WMH. In this analysis, the volume of WMH was measured at one year follow-up in the same group of AD patients. While overall WMH did not show a significant increase in volume at one year follow-up, the volume of DWH was significantly greater, specifically in the frontal lobes, suggesting that this region may be more susceptible to the effects of cerebrovascular disease, in keeping with previous research (Pugh et al., 2002). If WMH increase at a greater rate in this region compared to other brain regions, it may be that the PVH hyperintensities in frontal regions eventually connect to the DWH causing the common large confluent lesions extending from the frontal horns seen in many patients (Sachdev et al., 2005; Wen et al., 2004; DeCarli et al., 2004). Further long term follow-up studies are required to investigate whether these hyperintensities are in fact two separate entities that join together due to proximity.

An innovation of this study was the volumetric measurement of progression of lacunar infarcts. Lacunes were measured by Lesion Explorer, which allowed them to be distinguished as lesions within existing WMH, isointense with cerebrospinal fluid (CSF) on T1-weighted images. As a result, lacunes that would have been missed because they were not distinguishable as such on T2- or proton-density (PD)-scans were detectable. Lacunar volumes were found to significantly progress in the middle inferior frontal region. Although the largest baseline lacunar volumes were found in the basal ganglia, the frontal regions seemed to be preferentially susceptible to new lacunar infarcts over
one year follow up. This finding is in keeping with recent data from a large multi-centre study that used visual rating scales to measure lacunar progression (Gouw et al., 2007). The current analyses also revealed an unexpected relationship between the progression of DWH and decline in cognitive function. DWH progression was associated with a decline in tasks associated with verbal fluency, concept formation and initiation, thought to be related to frontal functioning. The current results lend some support to the theory that small vessel CVD is associated with the dysexecutive syndrome, potentially providing new treatment targets aimed at modifiable cardiovascular disease risk factors for patients with focal hyperintensities exhibiting executive dysfunction.

The presence of WMH in both normal aging and disease groups are receiving more attention. However, many methodological issues have arisen in both the measurement and the classification of WMH. The current study attempted to address some of these issues by using a combination of methodologies that have not been employed by previous studies. First, we specifically selected only probable and possible AD patients, and not VaD patients. This sample was free of overt cerebrovascular disease, including stroke and transient ischemic attack (TIA) and did not have any history of significant concomitant illness at baseline. Patients were screened to exclude such disease because the goal was to understand the topography and progression of WMH in patients with a history and clinical findings “typical” for AD. Although many large multi-centre studies are currently conducting extensive research on WMH, most involve a mixture of normal controls and cognitively impaired patients with differing histories. The patients in this study were chosen to represent a spectrum of white matter disease in a mild-moderate AD sample.
This study deliberately included analyses based on both a visual rating scale and volumetric measurement of WMH. The first study involved the use of a rating scale to stratify patients into groups based on severity of WMH. This distinction was then used to examine the relationship between WMH severity and brain perfusion patterns using SPECT, which was collected as part of the clinical work-up in our centre. In the attempt to separate patients into three distinct groups of differing WMH severity, we discovered that we had to screen many AD and allow at least one hyperintensity in order to generate a sample of patients with minimal or mild WMH, as such patients are relatively rare. This observation is in keeping with reports from other centers suggesting that small vessel disease is quite common in AD (Anonymous, 2001; Pantoni et al., 1995).

The introduction of a novel, semi-automatic volumetric software specifically designed to measure the extent of WMH (Levy-Cooperman, Ramirez, Lobaugh, & Black, 2007b), complemented by a standardized regional parcellation technique (semi-automated brain region extraction -SABRE) (Dade et al., 2004) was a methodological strength of this study, as it allowed new insights into topographical distribution. Lesion Explorer provides reliable measurements of WMH, separated into DWH, PVH and lacunes throughout the brain (Levy-Cooperman et al., 2007b). By eliminating the dependency on visual rating scales, we reduced the potential problem with sensitivity encountered when such scales are used to measure the progression of WMH (Kapeller et al., 2003). Lesion Explorer also generated continuous quantitative data, which allowed for more powerful correlational analyses as opposed to using arbitrary cut-off points for group comparison. The combination of Lesion Explorer and SABRE allowed us to establish the regional patterns of WMH, cross-sectionally and over time. In the case of
lacunes, we concluded that a larger volume at baseline does not necessarily relate to the susceptibility for progression. Baseline lacunes were found in the largest quantity in the basal ganglia region; however, they tended to show the greatest volume increase in the frontal regions at follow-up. Other methods which simply measure total brain WMH volume or parcellate into large brain regions cannot elicit such specific findings (Schmidt et al., 2003a; Sachdev et al., 2007).

While the methodologies employed in the current study are reliable and innovative, several limitations should be noted. First, the sample size in the current study was relatively small and unfortunately, at one year follow-up, 45% of patients had dropped out either due to illness, death or inability to complete cognitive testing, which decreased the sample size significantly, and could have introduced survival bias. In fact, when we compared patients with and without follow-up scans, the patients with follow-up were much healthier overall at baseline. In general, they had fewer cerebrovascular risk factors and scored higher on their neuropsychological testing. It should be noted that the size of the initial sample was limited by the strict exclusion criteria. Since AD is a heterogeneous disease which can present atypically, this sample may not be representative of all AD patients. An important issue that was not addressed in the current study was the influence, if any, of concomitant medications on WMH volumes and performance on cognitive tasks within this AD sample. A recent study investigated the anticholinergic activity of medications commonly used by older adults (e.g. antipsychotic, cardiovascular) and found that of 107 different drugs tested, 39 showed anticholinergic activity (Chew et al., 2008). These medications have been shown to be negatively correlated to performance on cognitive tasks (Chew, Mulsant, & Pollock, 2005). In fact, one study specifically
investigated the relationship between cognitive dysfunction and use of anticholinergic medications in elderly controls with WMH. They reported that WMH volume and the use of anticholinergic medication interacted synergistically, such that the cognitive impairment seen in patients with WMH was exacerbated by the presence of anticholinergic medications (Nebes et al., 2005). Additional data collection is currently underway which will include the concomitant medication profiles of this sample of AD patients. Further in-depth analyses accounting for the use of medications known to show anticholinergic activity and the relationship with WMH and cognition in AD is warranted.

A possible limitation of our imaging analysis was the basis on which PVH and DWH were classified. In this study, anything that touched the lateral ventricles in three-dimensional space was labeled as a PVH, and all other hyperintensities were labeled DWH. One argument against this labeling technique involves the measurement of progression of WMH. If PVH and DWH eventually join together, they will all be labeled as PVH, when in fact it is possible that the DWH expanded inward toward the ventricle as opposed to the PVH growing outward. This did not turn out to be the case, however, over the one year follow-up of our sample, though this could be an issue with a larger sample followed over a longer period. Labeling hyperintensity “subtypes” has yet to be standardized and results from the current study may not be comparable to other groups because they may measure the locations of PVH and DWH differently. For example, certain studies measured PVH as hyperintensities found within a specific measured area surrounding the ventricle (DeCarli, Fletcher, Ramey, Harvey, & Jagust, 2004). However,
we would argue that our three-dimensional technique is in many respects more objective and less arbitrary.

Many similar studies have used fluid attenuated inversion recovery (FLAIR) imaging to detect and measure WMH, and as a result, our study may not be comparable to others that have conducted volumetrics based solely on FLAIR (Admiraal-Behloul et al., 2005). FLAIR imaging can be used as an alternative to PD/T2 but is not necessary when the T1-weighted image is acquired. In addition, the PD/T2 acquisitions in combination with the T1 provide the ability to discriminate Virchow-Robin spaces from lacunes, which FLAIR imaging alone could not. FLAIR imaging may also underestimate thalamic lesions. On the other hand, FLAIR imaging provides better visual contrast for WMH, and may yield somewhat different WMH morphology when compared to PD/T2 sequences, and the debate as to which sequence is more pathologically relevant remains unresolved. It would have been beneficial to include both imaging sequences in the current study primarily for comparison purposes, but FLAIR was not available at the time this study started.

Subcortical Ischemic Vascular disease is complex, and debate exists over the etiology, the measurement and the classification of various aspects of the disease. First, standards for the measurement and distinction between PVH and DWH are needed so that research results are more consistent between research groups. A comparison of the many different measures of WMH across different centers would be a good starting point and would likely highlight the need for standardization. Unless standardization is achieved, it will be difficult to confirm, compare or contrast results from one study to another.
Another goal of future research should be long term longitudinal follow-up on the progression of lacunar infarcts. Some groups have investigated the progression of WMH at three- and six- year follow-up, but none have reported on the progression of lacunes, specifically lacunes within regions of WMH. Similar studies in large groups are needed to establish the natural course over time of WMH and lacunes using volumetric techniques as opposed to visual rating scales.

Recent studies have begun to introduce innovative imaging techniques to investigate WMH in normal elderly and cognitively impaired patients. Magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI) have been introduced to examine the chemical content of brain tissue and to measure diffusion along white matter tracts, respectively (Firbank, Minett, & O'Brien, 2003; Capizzano et al., 2000; Raz & Rodriguez, 2006). One of the most intriguing findings of MRS research is the detection of increased choline/creatinine ratios in the normal appearing white matter of individuals with WMH, which has been interpreted to implicate increased cell membrane turnover (Firbank et al., 2003). This finding, coupled with the fact that normal aging is associated with a relatively specific reduction in prefrontal metabolism, suggests that the pathological process involved in WMH may begin relatively early in life, perhaps even in middle age (Kuo et al., 2004). Since WMH are potentially preventable, interventions that address risk factors for the development of small as well as large vessel cerebrovascular disease may be very helpful in preventing disability in the elderly in future.

One final area that requires further study is investigation of variations in WMH topography between disease groups, preferentially within a large population study. It is
important to establish whether patterns of WMH differ between individual with AD, VaD, mixed disease, normal elderly and other degenerative diseases. Some research has examined the overall WMH volume differences between the different groups, but specific patterns have yet to be established, if they do indeed exist. By examining differing patterns in larger groups, it may be possible to then correlate the topography to different behavioral or cognitive deficits. Furthermore, this type of research may shed light on the differences, or lack thereof, between DWH and PVH. One possibility is that different topographical patterns of WMH are related to the range of disease comorbidity that includes AD, SIVD, and VaD. It may be that within this continuum, large PVH fall closer to the AD side of the continuum and as a result are more strongly correlated to the severity of MTL atrophy, while DWH fall closer to the VaD side (tend to progress more rapidly in frontal regions), and as a result are associated with greater declines in executive function.

Future endeavors that focus on the standardization of measurement tools and the relevance of co-morbidity with degenerative diseases could potentially help prevent late-life disability and functional impairment by treating modifiable cardiovascular disease risk factors that lead to white matter damage. The use of regional brain volumetry, including lesion analysis, may be a way to monitor effectiveness of intervention, as has been done in Multiple Sclerosis. These treatments, coupled with AD medication, may provide a better outcome for the population of elderly individuals afflicted with one or both diseases. By focusing on those individuals at risk due to family history, lifestyle or other risk factors, it may be possible to slow or possibly even halt the cognitive and functional decline associated with both Alzheimer’s and Cerebrovascular disease.
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APPENDIX: OTHER PUBLICATIONS

Frontal Lobe Hypoperfusion and Depressive Symptoms in Alzheimer’s Disease

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Research Article

Keywords: Alzheimer’s Disease; depressive symptoms; SPECT; MRI

Number of words in Abstract: 249

Number of words in Text: 4675

Number of Tables: 3

Number of Figures: 3

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Background: Depressive symptoms of varying severity are prevalent in up to 63% of Alzheimer’s disease (AD) patients and often result in greater cognitive decline and increased caregiver burden. The current study aimed to determine the neural correlates of depressive symptoms in a sample of AD patients using $^{99m}$Tc-ECD single photon emission computed tomography (SPECT). Methods: Fifty-six patients who met criteria for Probable AD were assessed using the Cornell Scale for Depression in Dementia. SPECT data was analyzed using an MRI-derived Region of Interest (ROI) anatomical template before and after atrophy correction, and statistical parametric mapping (SPM). Four frontal ROI’s were investigated bilaterally: 1) middle frontal gyrus (Brodman’s area [BA] 46), 2) orbitofrontal cortex (BA 11), 3) superior prefrontal (BA 8/9), and 4) anterior cingulate (BA 24/25/32/33). Results: Depressive symptoms were present in 27 of the AD patients (48%). Patients with depressive symptoms present (DSP) showed less perfusion in the right superior and bilateral middle frontal gyri (p < 0.005), left superior frontal (p < 0.05) and anterior cingulate gyri (p < 0.005) before atrophy correction. SPM analyses revealed significantly lower perfusion in bilateral dorsolateral and superior prefrontal cortex of DSP patients (Right - p < 0.005; Left -p < 0.05). SPECT ROI analyses with atrophy correction revealed similar trends as non-atrophy corrected data but did not reach statistical significance. Conclusion: In this study, depressive symptoms in AD patients were associated with relative hypoperfusion in the prefrontal cortex when compared to AD patients’ without depressive symptoms. These findings are consistent with previous reports in primary depression that suggest that these regions are involved in affect and emotional regulation.
Depressive symptoms of varying severity are prevalent in up to 63% of patients with AD (1), and when present, can result in more rapid cognitive decline and increased caregiver burden (2;3). Although both the psychiatric and neurological literature acknowledge the occurrence of affective and psychotic features in patients with AD, very little is known about the underlying mechanisms of depressive symptoms in this patient population. Certain studies have emphasized the psychosocial factors in AD such as functional and cognitive disability, while others have stressed the neurobiological underpinnings (4). As in primary depression, it is likely that depressive symptoms in AD are multifactorial. Abnormalities in limbic-frontal circuitry have been associated with depressive symptoms in non-demented subjects. Known substrates of executive function, such as the dorsolateral prefrontal region, have consistently shown abnormally reduced baseline function in clinical depression (5). The etiology is not well understood, but a neurobiological rather than reactive phenomenon seems likely. Mayberg et al. (2001) proposed a working model of primary depression which involves three interconnected frontal regions: the dorsal, ventral, and rostral cingulate. The dorsal compartment is thought to be involved in the cognitive aspects of negative emotion such as apathy, impaired attention and dysexecutive syndrome, while the ventral compartment may mediate the circadian and vegetative aspects of depression such as disturbed sleep and appetite. The rostral cingulate mediates interactions between the dorsal and ventral cortical subcortical pathways (6). Non-demented subjects in the depressed state show hyperactivation of the anterior cingulate, particularly sub-genual cingulate area 25 with hypoactivation of dorsolateral prefrontal cortex (7) a pattern that is modified when there
is an antidepressant treatment response (8). How these regions may be involved in depressive symptoms associated with AD has yet to be fully elucidated.

Single photon emission computed tomography (SPECT) studies have been useful in furthering knowledge of brain-behavior relationships in AD, but, few studies have investigated possible regional perfusion correlates of depressive symptoms in AD. One HMPAO-SPECT study reported selective hypoperfusion in the bilateral anterior and posterior cingulate gyri and precuneus in depressed AD patients using Statistical Parametric Mapping (SPM) (9). A longitudinal HMPAO-SPECT study of dementia and depression reported an increase in perfusion in the right cingulate gyrus and right cerebellum in dementia patients who were significantly less depressed at two-year follow-up, but this study only included ten subjects with varying types of dementia (10). Similarly, a $^{18}$F-fluorodeoxyglucose positron emission tomography (PET) study reported decreased activity in the superior frontal cortex bilaterally and the left anterior cingulate gyrus in eight AD patients with depressive symptoms (11).

The diagnosis of depression in AD patients is not straightforward. Research suggests that the reported frequency of depression varies substantially with both the method of assessment and the person being interviewed (12). For example, cognitive impairment in AD patients may limit their ability to report depressive symptoms to clinicians (3), while caregivers often over-report depressive symptoms in the patient (13). Another challenge has to do with the assessment tools used for depression in AD patients. Most traditional depression scales were not designed for use with demented patients, or even with elderly subjects (14;15). The Cornell Scale for Depression in Dementia (CSDD) was specifically designed for use in the dementia population and is based on combined caregiver and
patient interviews (14). Recent studies suggest that the CSDD shows promise in the detection, quantification and management of depression in AD (3;12;16).

The current study aimed to investigate regional perfusion correlates of depressive symptoms in a sample of AD patients using SPECT imaging and the CSDD. Specifically, it was hypothesized that depressive symptoms in AD would be associated with decreased perfusion ratios in the areas of the superior, middle and orbitofrontal and anterior cingulate regions, postulated to be involved in the cognitive-behavioral circuitry for depressive states. We investigated this relationship using three different techniques. We first examined the SPECT data using an ROI analysis based on an MRI-derived anatomical template. Due to the presence of brain atrophy in many patients, we included an analysis of SPECT data with atrophy correction of perfusion values using estimates of brain tissue volume measured by magnetic resonance imaging (MRI). Statistical parametric mapping (SPM) was also utilized in order to confirm ROI findings. SPM examined total cerebral blood flow distribution differences between the two groups in the entire brain and not just specific ROIs.

METHODS

Participants

The study patients were recruited from a referral-based outpatient Memory Clinic at the Sunnybrook Health Sciences Centre, a University of Toronto academic health care institution, as part of the Sunnybrook Dementia Study, a longitudinal observational neuroimaging study of AD and other dementias. All patients were fluent in English and had adequate visual and auditory acuity to complete neuropsychological testing. As part of the longitudinal study, patients received a comprehensive clinical evaluation, including
detailed medical history, neurological examination, routine laboratory investigation and neuropsychological testing with a standardized test battery. The presence of cerebrovascular risk factors was ascertained including: arterial hypertension, diabetes, hyperlipidemia, and ‘other cardiac disorders’ such as coronary artery disease. Patients were consecutively enrolled and were included in the current study if they met the following criteria. Patients in this study had historical profiles compatible with AD, with insidious onset and gradual decline. All patients in this study met the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer’s Disease and Related Disorders Association criteria for probable or possible AD(17), and the Diagnostic and Statistical Manual of Mental Disorders (fourth edition) (18) criteria for dementia. A final inclusion criteria was that participants SPECT, MRI and neuropsychological testing was completed within a consecutive 3 month period. Patients were excluded if they had a history of significant head trauma, psychotic disorder unrelated to dementia, psychoactive substance abuse, major depressive disorder or any other psychiatric or neurological illness.

Depressive symptoms were assessed using the CSDD (14). The CSDD is a 19-item, clinician administered depression scale developed to measure depressive symptom severity in older adults with dementia. Significant depressive symptoms were based on a score of 8 or more on the CSDD (range 0 to 38). Patients were also rated on the severity of white-matter hyperintensities (MRI scans) noted to be associated with depression and dementia (19). White matter hyperintensities were rated according to the Age-Related White Matter Changes Scale (ARWMC) a visual rating scale with good interrater reliability and face validity (20).
Fifty-six patients with AD were included in the study (25 men and 31 women; mean age, 76.8 ±6.2 years, see Table 1). Mini-mental Status Exam score for all participants was 23.6 ±4.0, showing mild impairment. Twenty-seven patients showed evidence of depressive symptoms with scores greater than or equal to eight on the CSDD (48%; Range: 8 - 24). In the DSP group, the CSDD score was 8 - 10 in nine patients, 11 - 13 in nine patients, 14 - 16 in five patients, and 18 - 24 in seven patients.

*SPECT Scans and Regional Perfusion Ratios*

SPECT imaging was performed using a triple-head gamma camera (Prism 3000XP; Phillips Medical Systems Inc, Cleveland, Ohio), a minimum of 30 min and a maximum of 120 min after injection of 20 mCi (740 MBq) of the radiopharmaceutical Technetium-99m ethyl cysteinate dimer (99mTc-ECD). Each view consisted of a 128x128 pixel image with a typical reconstructed image resolution of 9.7 mm full-width at half-maximum. Total imaging time was 19 minutes. Reconstruction was performed using a ramp-filtered back-projection algorithm followed by a 3-dimensional restoration post-filter (Wiener filter, multiplier 1.0). Ellipses were fit to the approximate location of the outline of the head in each transaxial image and a calculated attenuation correction applied (21). Reconstructed images were coregistered to a SPECT template, which was an average of 28 healthy elderly control scans. A T1-weighted MRI, whose dimensions were similar to the SPECT template, was the source of 79 bilateral regions of interest (22). To obtain ROI intensity values, a common transformation was used move each SPECT from SPECT template space into MRI space. Because the cerebellum is frequently used to normalize SPECT counts(23) (24), mean perfusion in regions of
interest was referenced to the cerebellum to provide semi-quantitative measures of regional perfusion.

*Regions of Interest*

Prefrontal and anterior cingulate cortices were chosen as target regions hypothesized to be adversely affected by depression. The prefrontal regions included the middle frontal gyrus (Brodmann’s area [BA 46], orbitofrontal cortex (BA 11), and superior prefrontal (BA 8/9) regions; the anterior cingulate ROIs included the combined dorsal (BA 25) and middle (BA 24/32/33) regions. Three additional ROIs, were assigned as control regions to investigate perfusion in areas typically affected in AD: posterior cingulate (BA 23/31), angular gyrus (BA 39), and superior parietal (BA 7) (25) (26).

**MRI**

*MRI Acquisition*

All brain images were acquired using a 1.5 T Signa MR imager (GE Medical systems, Milwaukee, WI). Three image sets were acquired in the same imaging session: T1-weighted (axial 3D SPGR with 5ms TE, 35ms TR, 35° flip angle, 1 NEX, 22 x 16.5 cm FOV, 0.859 x 0.859mm in-plane resolution, and 1.2 to 1.4mm slice thickness), proton-density (PD) and T2-weighted images (interleaved axial spin echo, with TEs of 30 and 80 ms, 3s TR, 0.5 NEX, 20 x 20cm FOV, 0.781 x 0.781mm in-plane resolution, and 3mm slice thickness).

*MRI-Based Pixel -by- Pixel Partial -Volume Correction*

A previously described computerized MRI-based partial volume correction (PVC) algorithm originally developed for PET was modified for use with SPECT images (27). The algorithm compensates for the limited spatial resolution of the SPECT images by using higher-resolution segmented MR data to determine the fractional contributions of
anatomic structures to observed SPECT voxel activities. MR brain extraction and automated tissue segmentation was accomplished using a modified version of previously described methods (28). This is a multi-spectral approach which uses the PD/T2 images for brain extraction, followed by a T1-based segmentation where local intensity histograms are fitted to four Gaussian curves to derive a cut-off used to classify each voxel as white matter (WM), grey matter (GM), or cerebrospinal fluid (CSF). Following the procedure of Bencherif et al, binary WM, GM and CSF masks were generated from the segmented MR and smoothed by the estimated point spread function of the SPECT scanner. The smoothed WM and CSF masks were thresholded to obtain masks containing only pixels which were members of the tissue type in question with high probability. SPECT data were interpolated to the same pixel dimensions as the MR image. The interpolated SPECT data were then co-registered to the MR image. The masks were applied to the co-registered SPECT data for calculation of mean WM and CSF uptake. This is a slight deviation from the original Bencherif algorithm, which called for co-registration of MR-PET (MR-SPECT) in PET (SPECT) space (i.e., down-sampling the higher spatial resolution MR images to PET (SPECT) pixel dimensions during co-registration and applying the corrections in PET (SPECT) space). The current calculations were performed in higher-resolution MR space for improved accuracy. The WM and CSF smoothed binary masks were then scaled by their respective mean uptake values and subtracted from the co-registered original SPECT to remove WM and CSF “spill-in” counts from GM voxels. Finally, the resulting image was divided by the smoothed GM mask to compensate for the “spill-out” of GM counts to surrounding tissues.
Image Analysis by Statistical Parametric Mapping

Data analysis was also conducted using statistical parametric mapping (SPM) software version 2 (29) (30) running under the MATLAB Version 7 on Windows XP. Unlike the ROI method, SPM does not analyze data using predefined regions. Instead, it performs unbiased voxel by voxel analysis of the entire brain image and produces a visual representation of the results. Images in each group were normalized into approximate Talairach space by transforming them into Montreal Neurological Institute (MNI) space. After the completion of the normalization, the images were also individually checked to detect normalization errors. Subsequently, the images were smoothed using an isotropic Gaussian kernel of 12mm FWHM to increase the signal-to-noise ratio. Each image was scaled to 50 using proportional scaling option, which minimizes intersubject variability. SPECT images of depressed and non-depressed groups were compared using independent t-test option, and the height threshold was set to less than 0.01 and 0.05 to produce two sets of results for comparison with the results obtained with ROI method. Only cluster level p-values, corrected for multiple comparisons, of less than 0.05 were considered significant. Since the co-ordinate system of Talairach and Tournoux is not exactly the same as that of the MNI, we converted MNI coordinates into Talairach (Talairach and Tournoux, 1988) coordinates utilizing mni2tal script (http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml)(31). In addition, we visually inspected the location of areas of significant change in perfusion in MNI space by superimposing them on Colin27 MRI, which is also normalized to MNI space.

Statistical Analysis
Data analysis was performed using SPSS software version 11.0 (SPSS Inc, Chicago, Ill). To explore whether the groups differed significantly on demographic measures, an analysis of variance (ANOVA) was performed on continuous variables that included age and years of education. Categorical variables, such as gender, were compared using Chi-square ($\chi^2$) analysis. Mean perfusion ratios in patients with depressive symptoms present (DSP) and patients with depressive symptoms absent (DSA) were compared using multivariate analysis of variance (MANOVA) for both the uncorrected and atrophy corrected data sets. To assess the relationship between each ROI and the severity of depression (measured by the CSDD raw score), Pearson’s correlation test was applied. The level of significance was $p < 0.05$.

Results

Patients and Behaviours

No significant differences were observed between the DSP and DSA groups with respect to age, cognitive status, sex, or level of education (Table 1). The CSDD score was not significantly correlated with age, sex, education level, duration of illness, or MMSE. The two groups were also compared on cerebrovascular risk factors, use of cholinesterase inhibitors or antidepressants, and severity of white matter disease. No significant differences were seen between the two groups on any of these measures (DSP vs. DSA). Four of the DSA patients were taking antidepressants (CSDD score, mean 3.3 ±2.9) and five DSP patients were taking antidepressants (CSDD score, mean 15.4 ±3.9).

ROI Analyses

Mean perfusion ratios in DSP and DSA groups are listed in Table 2. MANOVA results indicate a significant overall group difference on mean perfusion ($F_{(8,47)}=2.49, p = 0.02$).
Univariate analyses showed that DSP patients had significantly lower perfusion in the right superior ($F_{(1,54)}=11.3, p = 0.001; \text{Cohen’s } d = 0.9; r = 0.45$) and bilateral middle frontal gyrus (Left – $F_{(1,54)}=8.02, p = 0.006; \text{Cohen’s } d = 0.4, r= 0.21$; Right- $F_{(1,54)}=9.02, p = 0.004; \text{Cohen’s } d = 0.7, r = 0.32$). Decreased perfusion was also evident in the left superior frontal gyrus ($F_{(1,54)}= 4.12, p = 0.047$) but not of the same magnitude (Cohen’s $d = 0.4; r = 0.21$). Perfusion in the anterior cingulate bilaterally (Left – $F_{(1,54)}=7.88, p < 0.007; \text{Cohen’s } d = 0.8, r= 0.34$; Right- $F_{(1,54)}=9.22, p < 0.007\text{Cohen’s } d = 0.7, r = 0.35$) was also significantly lower in the DSP group (Figure 2). No significant perfusion differences were seen in the orbitofrontal cortex, but there was a trend toward decreased perfusion in the left orbitofrontal region ($F_{(1,54)}=3.41, p = 0.07; \text{Cohen’s } d = 0.4, r = 0.22$). Significant but weak negative correlations were seen between the CSDD raw score and perfusion ratios in the bilateral anterior cingulate ($r = -0.31, p = 0.02$ (left), $r= -0.28, p = 0.036$ (right)) and the right superior prefrontal region ($r= -0.28, p = 0.037$). Data were also analyzed with age, years of education and disease severity included as covariates in the model (see Table 2). With age and education included as covariates in the model, the group difference remained significant ($F_{(8,45)}=2.24, p < 0.05$) with lower perfusion in the bilateral frontal and cingulate gyri in the DSP group. The two groups did not differ significantly in any of the control ROIs (posterior cingulate, angular gyrus, and superior parietal).

**Partial Volume Correction Results**

Five subjects (DSP) from the original sample were excluded from the partial volume correction analysis because of poor MRI scan quality for a total of 51 subjects in the partial-volume corrected data set. MANCOVA controlling for years of education and
disease revealed a trend towards group differences in mean perfusion ($F_{(8,40)}=2.0, p=0.07$). Exploratory univariate analyses indicated similar patterns of decreased perfusion in the depressed group in the right superior ($F_{(3,47)}=5.15, p<0.05; \text{Cohen's d = 0.4, r = 0.23}$) and middle ($F_{(3,47)}=4.5, p<0.05; \text{Cohen's d = 0.5, r = 0.24}$) prefrontal cortex, and in the right ($F_{(3,47)}=4.68, p<0.05; \text{Cohen's d = 0.6, r = 0.27}$) and left ($F_{(3,47)}=5.09, p<0.05; \text{Cohen's d = 0.6, r = 0.29}$) anterior cingulate.

**SPM Whole Brain Analysis**

Comparison of the DSP and DSA groups using SPM analyses are shown in Figure 3. The coordinates of areas with significantly less perfusion in DSP are listed in Table 3 (Height threshold 0.01). The right dorsolateral and superior prefrontal regions, constituted the major regions of perfusion differences between the depressed and non-depressed groups ($p < 0.005$, corrected). Similar patterns were seen in the left dorsolateral and superior prefrontal cortex based on uncorrected $p$ values ($p < 0.05$). No significant differences were seen in the anterior cingulate region. No regions had a significant increase in perfusion in either group.

**Atrophy**

An atrophy ratio was calculated for the eight ROIs, comparing the number of voxels originally assigned to a specific ROI with the number of voxels in that same ROI after atrophy correction. Mean ratios for the eight ROIs were compared between the two AD groups. ANOVA did not reveal any significant differences in amount of atrophy between DSP and DSA groups. Atrophy ratios did not correlate with raw scores on the CSDD.

**Concomitant Medications**
Of the 56 participants, 18 were taking cholinesterase inhibitor (11 DSP vs. 7 DSA) at the time of their scan, the two groups were not significantly different based on Fisher’s exact tests (p > 0.05). Perfusion in the eight ROIs did not significantly differ in those individuals using cholinesterase inhibitors compared to those who were not (F_{(8,47)} = 0.94, ns) nor did perfusion differ in participants using antidepressants compared with those who were not (F_{(8,47)} = 1.07, ns). Four of the DSA patients were taking antidepressants (mean ±SD CSDD score, 3.3 ±2.9) and five DSP were taking antidepressants (mean ±SD CSDD score, 15.4 ±3.9). An additional analysis excluding all subjects on antidepressants (N=47) revealed similar findings. Although the sample size was smaller, univariate analyses showed that DSP patients had significantly lower perfusion in the right superior (F_{(1,45)}= 6.8, p = 0.01) bilateral middle frontal gyrus (Left – F_{(1,45)}= 5.5, p = 0.02; Right- F_{(1,45)}= 5.3, p = 0.03 ) and anterior cingulate bilaterally (Left – F_{(1,45)}= 4.8, p = 0.03; Right- F_{(1,45)}= 5.3, p = 0.02). Furthermore, three patients in the DSP group were taking PRN benzodiazepine (Lorazepam) and two were on small doses of Risperidone. Two patients in the DSA group were also taking PRN benzodiazepine (1 Lorazepam, 1 Alprazolam).

_Apathy_

Apathy includes symptoms such as reduction or lack of interest, productivity, will, initiative and affective response toward positive or negative events (32;33). Apathy is also the most frequent behavioral symptom in Alzheimer’s disease and can often co-occur with depression (34;35). It has been associated with decreased perfusion in the orbito- and middle frontal (36) and the anterior cingulate regions (32). In order to examine potential effects of co-existing apathy in our sample, the relationship between apathy sub-score on the Neuropsychiatric Inventory (NPI) (12) and perfusion in the 8
ROIs was investigated. NPI scores were available for 37 of the 56 patients. Both the depression (r =0.65, p < 0.001) and apathy (r =0.52, p < 0.005) sub-scores were highly correlated with the CSDD. No significant correlations were found between the apathy subscore and perfusion in the ROIs.

Discussion

The current study suggests that when compared to AD patients without depressive symptoms, AD patients with depressive symptoms have relative hypoperfusion in the prefrontal cortex. Both ROI and SPM analyses showed decreased perfusion in the bilateral superior frontal and middle frontal cortices corresponding to BAs 8/9 and 46 respectively. Differences in atrophy were examined by correcting for tissue loss in the sample. A group comparison suggested no difference in atrophy between the two groups, nor was the amount of atrophy related to depression scores. It is possible that two components may be contributing to the relative hypoperfusion seen in AD patients with depressive symptoms. There is a structural component related to an actual loss of tissue in the ROIs, which was seen equally in both groups, and a functional component indexed by the lower perfusion seen on SPECT in the DSP group. It may be that subtracting out the structural component reduced sensitivity in the sample size we had available.

These imaging findings are consistent with previous reports in primary and secondary depression studies that suggest that these regions, along with the hippocampus and amygdala may be involved in affect and emotional regulation (5;37;38). As for the anterior cingulate, the ROI group comparison indicated significantly lower perfusion in AD patients with depressive symptoms, but we could not confirm this finding with SPM analysis. No relationship between the orbitofrontal cortex and depressive symptoms was
evident in the current study even though this region has been implicated in major
depression studies (39).

Relationships were further investigated using correlation analyses, which
suggested an inverse correlation between CSDD (higher score indicates a greater severity
of depressive symptoms) and perfusion in the superior prefrontal and anterior cingulate
regions, in keeping with studies that have investigated depressive symptoms in AD. A
previous study of AD reported an association between the presence of depressive
symptoms (n=19/53, 33%), defined as depressed mood including melancholia and
sadness, and decreased normalized glucose metabolic rates in the superior frontal and left
anterior cingulate cortices bilaterally (11). That study assessed depressive symptoms
using the NPI- subscale for depression. The NPI scale, while advantageous in that it
quantifies depression in the context of other neuropsychiatric symptoms, lacks sensitivity
and has a limited range for quantifying depressive symptoms (3). A similar study
examined perfusion differences between AD patients with and without depression using
the Hamilton Depression Rating Scale and HMPAO-SPECT imaging using SPM
analysis. It found perfusion differences in the anterior cingulate and precunei bilaterally
in a small group of depressed AD patients (n=8) when compared to non-depressed
patients (n=35) (9). Our study differs from this study in some important details. First,
we used the CSDD (14), a rating scale specifically designed for elderly patients with
dementia, which has been shown to have high interrater reliability, internal consistency
and sensitivity (2;3). Our study is among the first to incorporate the use of the CSDD,
which may be more sensitive for detecting depressive symptoms in an AD population
(16). However, it is currently being used in a National Institute of Mental Health clinical trial investigating treatment for depressive symptoms in AD.

The previous study did not provide adequate detail as to the method used to dichotomize patients based on Hamilton Depression Rating scale scores. Although patients were also interviewed by a psychiatrist, this was not used to assess the presence and severity of depression. We also used a novel quantitative ROI analysis method (22) in addition to SPM analysis (30) to test whether frontal regions chosen a priori would show lower perfusion in the DSP versus DSA groups. Lastly, our results are based on a larger sample of AD patients with depressive symptoms (n= 27) in comparison to the previous studies which included a maximum of 19 patients with depressive symptoms.

Hypoperfusion of the bilateral anterior cingulate has previously been reported in AD patients with apathy (32) and depression (9). Conversely, hyperactivation of the anterior cingulate has been reported in non-demented subjects in the depressed state (8). In one study, hyperactivation on EEG analysis, of Brodmann’s areas 24 and 32 in pre-treatment phase predicted favorable treatment response to antidepressants, suggesting that hyperactivation in the rostral cingulate may represent a compensatory reaction to depression (40). In the current study, anterior cingulate perfusion differences were found when using the ROI methodology, but were not reproduced using SPM analysis with a threshold level of p < 0.01. It should be noted that when the threshold level was lowered (p < 0.05) the anterior cingulate and the insula did differ between the two groups. These results and those of previous studies support the working model of primary depression which suggests that the rostral cingulate plays a key role in integrating the dorsal and
ventral compartments and acts as a gateway through which limbic motivation influences
goal-directed behavior (7;32;40).

The current study also supports a greater role for the right hemisphere in
depressive symptoms associated with AD. Both SPM and ROI analyses revealed a
stronger relationship between lower right hemisphere perfusion and depressive
symptoms. In general, depression is reported with lesions of either frontal lobe, while
mania is more often reported with lesions to right frontal lobe. Left hemisphere and
specifically left frontal damage has been associated most commonly with depressed mood
with subjects showing a dysphoric reaction manifested by feelings of despair,
hopelessness and anger (41). Whether depressive symptoms can follow right frontal
lesions is more controversial. Some investigators have found depression related to lesions
in the right frontal area (42), but these patients generally have more psychological
symptoms overall, including irritability, loss of interest, and difficulty in concentration.
Apathy and depression are both common behavioral symptoms associated with AD;
therefore, right hemisphere involvement may reflect the co-occurrence of two common
behavioral symptoms in this group of patients. We attempted to address the co-
occurrence of apathy in our sample using the apathy sub-score of the NPI (43). Apathy
score did not correlate with perfusion; however, the limited range for scoring apathy on
the NPI may reduce sensitivity with this sample size. More detailed assessment of apathy
and depressive symptoms, using structured interviews, in a larger AD sample should be
explored in further studies.

Depressive symptoms were present in 48% of AD patients. These results are
consistent with previous studies that have used a variety of different rating scales to
assess depression in dementia (2;3). Participants in this sample did not differ on the presence of cerebrovascular risk factors or white matter hyperintensities, two factors that can be associated with depression in dementia (44;45). The current study included only AD disease patients: additional research that includes a sample of healthy, age- and sex-matched elderly controls would provide additional information on the extent to which depressive symptoms in AD correlate with perfusion deficits when compared to a healthy sample. In addition, subjects in the two groups were not compared based on a clinical scale for severity of dementia, and as a result it is possible that patients in the two groups could have had different dementia severities, that was not picked up by their performance on the MMSE. It should also be noted that participants in this study did not receive a formal psychiatric assessment using a structured interview; therefore, a formal diagnosis of depression based on DSM-IV-TR criteria could not be confirmed. The current study focused on the correlates of depressive symptoms in AD, which are specific and can effect functioning in both minor and major depression (46;47). Therefore, depressive symptoms seen in AD may be significant regardless of whether a patient meets criteria for major depressive disorder. While there has been some suggestion that depressive symptoms in AD are a psychological reaction to the knowledge of having the disease, these findings support the possibility that the neuropathological processes involved in AD play a role in behavioral as well as more commonly reported cognitive dysfunction.

ACKNOWLEDGMENTS

We acknowledge the Government of Ontario/Paul and Adelle Deacon Graduate Scholarships in Science and Technology, the L.C. Campbell Foundation, the Canadian
Institutes of Health Research, Alzheimer’s Society of Canada, and the Alzheimer’s Association for their support. We are also grateful for the assistance received from Isabel Lam and Isabelle Guimont with data collection and database entry. We also thank Christopher Scott and Mario Masellis for their review and helpful comments.

FINANCIAL DISCLOSURES

The authors report no conflict of interest, financial or otherwise related to this manuscript.
Table 1. Demographic characteristics of the sample (N=56)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DSP (n=27)</th>
<th>DSA (n=29)</th>
<th>Group Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women*</td>
<td>9/18</td>
<td>16/13</td>
<td>$\chi^2 = 2.69$  p = 0.10</td>
</tr>
<tr>
<td>Age, y†</td>
<td>78.3(7.2)</td>
<td>75.5(4.8)</td>
<td>$F_{(1,54)} = 2.98$, p = 0.09</td>
</tr>
<tr>
<td>MMSE†</td>
<td>23.2(4.4)</td>
<td>24.0(3.6)</td>
<td>$F_{(1,54)} = 0.53$, p = 0.47</td>
</tr>
<tr>
<td>YOE†</td>
<td>13.4(3.0)</td>
<td>13.8(4.0)</td>
<td>$F_{(1,54)} = 0.14$, p = 0.71</td>
</tr>
<tr>
<td>CVD risk factors, N (%)*</td>
<td>17(64%)</td>
<td>15(52%)</td>
<td>$\chi^2 = 0.72$  p = 0.40</td>
</tr>
<tr>
<td>Antidepressants, N (%)</td>
<td>5(19%)</td>
<td>4(14%)</td>
<td>$\chi^2 = 0.23$  p = 0.63</td>
</tr>
<tr>
<td>Cholinesterase Inhibitor, N (%)*</td>
<td>11(47%)</td>
<td>7(24%)</td>
<td>$\chi^2 = 1.76$  p = 0.18</td>
</tr>
<tr>
<td>ARWMC Scale†</td>
<td>9.5(6.1)</td>
<td>6.7(6.1)</td>
<td>$F_{(1,54)} = 3.02$, p = 0.09</td>
</tr>
<tr>
<td>CSDD†</td>
<td>13.3(4.5)</td>
<td>3.9(2.1)</td>
<td>$F_{(1,54)} = 102$, p &lt;0.001</td>
</tr>
<tr>
<td>CSDD Range</td>
<td>8-24</td>
<td>0-7</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean (standard deviation).

† One Way Analysis of Variance (ANOVA); * Chi Square analysis
DSP = Depressive Symptoms Present; DSA = Depressive Symptoms Absent
MMSE = Mini-Mental State Examination (48); CVD = Cerebrovascular Disease;
ARWMC = Age-Related White Matter Changes Scale (Wahlund et al 2001); YOE = Years of Education
CSDD = Cornell Scale for Depression in Dementia (Alexopoulos et al 1988)
Table 2. Regional perfusion ratios in the DSP vs. DSA groups in four bilateral regions of interest

<table>
<thead>
<tr>
<th>Region</th>
<th>DSP</th>
<th>DSA</th>
<th>Group Main Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbitofrontal cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.75(0.07)</td>
<td>0.78(0.06)</td>
<td>F(1,54) = 3.41, P = 0.07</td>
</tr>
<tr>
<td>Right</td>
<td>0.76(0.07)</td>
<td>0.79(0.06)</td>
<td>F(1,54) = 1.57, P = 0.21</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.72(0.07)</td>
<td>0.79(0.10)</td>
<td>F(1,54) = 7.88, P = 0.007**</td>
</tr>
<tr>
<td>Right</td>
<td>0.64(0.08)</td>
<td>0.70(0.08)</td>
<td>F(1,54) = 9.22, P = 0.004**</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.73(0.06)</td>
<td>0.76(0.08)</td>
<td>F(1,54) = 4.12, P = 0.047*</td>
</tr>
<tr>
<td>Right</td>
<td>0.68(0.06)</td>
<td>0.74(0.06)</td>
<td>F(1,54) = 11.3, P = 0.001**</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.82(0.07)</td>
<td>0.88(0.08)</td>
<td>F(1,54) = 8.02, P = 0.006**</td>
</tr>
<tr>
<td>Right</td>
<td>0.76(0.08)</td>
<td>0.81(0.07)</td>
<td>F(1,54) = 9.02, P = 0.004**</td>
</tr>
</tbody>
</table>

DSP = Depressive Symptoms Present; DSA = Depressive Symptoms Absent
Values are given as mean (SD). Perfusion ratios referenced to the cerebellum.
Based on Univariate Analysis of Variance.
Table 3. Results of Statistical Parametric Mapping analysis- regions of decreased perfusion in the DSP compared to DSA groups.

<table>
<thead>
<tr>
<th>Anatomical Region</th>
<th>Brodmann's Area</th>
<th>Coordinates MNI (Talairach)</th>
<th>p Value Uncorrected</th>
<th>p Value Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right superior frontal gyrus</td>
<td>46/8/9</td>
<td>32 (32) 16 (18) 62 (56)</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>Right middle frontal gyrus</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left superior/middle frontal gyrus</td>
<td>9</td>
<td>-45 (-45) 24 (25) 36 (32)</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>Left inferior frontal gyrus</td>
<td>44/45/46</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Height Threshold = 0.01.
Figure Legends

Figure 1. Frontal regions as defined by the Single - Photon Emission Computed Tomography (SPECT) region-of-interest template. (A) Superior Frontal Gyrus (Brodmann’s Area 8/9), (B) Middle Frontal Gyrus (Brodmann’s Area 46), (C) Orbitofrontal Cortex (Brodmann’s Area 11), (D) Anterior Cingulate Cortex (Brodmann’s Area 25/24).

Figure 2. Histogram comparing SPECT perfusion ratios (referenced to the cerebellum) in six frontal regions of interest (ROI) in depressive symptoms present (DSP) and depressive symptoms absent (DSA) patients.

Figure 3. Statistical Parametric Mapping projections showing areas with reduced cerebral perfusion in depressed compared with non-depressed patients (height threshold= 0.01).
Reference List


**Misclassified tissue volumes in Alzheimer’s Disease patients with white matter hyperintensities: Importance of lesion segmentation procedures for volumetric analysis.**

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Acknowledgments

The research reported in this article was supported by the K.M. Hunter Graduate Scholarships, the L.C. Campbell Cognitive Neurology Research Unit, the Canadian Institutes of Health Research, Alzheimer’s Society of Canada, and the Alzheimer’s Association. We would also like to thank Dr. Fuqiang Gao and Christopher Scott for their assistance in data collection.
Misclassified tissue volumes in Alzheimer’s Disease patients with white matter hyperintensities: Importance of lesion segmentation procedures for volumetric analysis.

Cover Title: Misclassified white matter tissue volumes in Alzheimer’s Disease
Keywords: MRI, Alzheimer’s disease, White matter hyperintensities

Word Count: 4853

Tables and Figures: 3 Tables, 4 Figures
Abstract

Background and Purpose: MRI based quantification of grey and white matter volume is common in studies involving elderly patient populations. The aim of the current study was to describe the effects of not accounting for subcortical white matter hyperintensities (WMH) on tissue volumes in Alzheimer Disease (AD) patients with varying degrees of WMH (mild: n=19, moderate: n=22, severe: n=18). Methods: An automated tissue segmentation protocol that was optimized for an elderly population, a brain regional parcellation procedure, and a lesion segmentation protocol were applied to measure tissue volumes (whole brain and regional lobar volumes) with and without lesion segmentation to quantify the volume of misclassified tissue. Results: After application of the tissue segmentation protocol and lesion analysis, mean total percentage misclassified volume across all subjects was two percent (17.9cm$^3$) of whole brain volume (corrected for total intracranial capacity). Mean percentage of misclassified tissue volumes for the severe group was 4.8% of whole brain, which translates to a mean volume 42.2cm$^3$. Grey matter volume was most overestimated in the severe group, where 6.4% of the total grey matter volume was derived from misclassified WMH. The regional analysis showed that frontal (41%, 7.4cm$^3$) and inferior parietal (18%, 3.25cm$^3$) lobes were most affected by tissue misclassification. Conclusion: MRI-based volumetric studies of Alzheimer Disease that do not account for WMH can expect an erroneous inflation of grey and/or white matter volumes, especially in the frontal and inferior parietal regions. To avoid this source of error, MRI-based volumetric studies in patient populations susceptible to hyperintensities should include a WMH segmentation protocol.
Introduction

Over the past twenty-five years, the availability of magnetic resonance imaging (MRI) has progressed to a point where clinicians and researchers have an array of sophisticated image-processing techniques at their disposal. In structural neuroimaging, automatic segmentation algorithms can be used to obtain volumetric information derived from voxel intensity differences with a set of multi-modal MRIs of the brain. Segmentation techniques vary in approach but generally provide the same information - tissue volumes for grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) \(^1\) \(^2\).

There are numerous ways of obtaining brain tissue volumes in structural neuroimaging. Probability map registration involves the use of templates (e.g. Talairach) in a software package\(^3\). The Statistical Parametric Mapping (SPM) package, uses a template-based approach where a scalar function of a spatially normalized image can be applied to groups of subjects, such as in voxel-based morphometry (VBM) techniques\(^4\). Mathematical modeling such as discriminant analysis \(^2\), \(^5\) or Gaussian curve fitting\(^1\), apply mathematical models to generate intensity ranges for each brain tissue type. Artificial intelligence/fuzzy logic algorithms (e.g. fuzzy c-means algorithm) involve procedures where class rules are generated by a blinded expert followed by an iterative clustering process where tissue labelling occurs based on the predefined class/tissue type rules \(^6\), \(^7\). Still others use combinations of approaches creating multi-agent approaches \(^8\) or pattern recognition approaches \(^9\).
One limitation of many automatic tissue segmentation procedures is that they are generally based on T1-weighted images. As such, they are not designed to account for the frequent presence of focal or diffuse signal changes seen on T2-weighted images as hyperintensities in the cerebral white matter of both asymptomatic and cognitively impaired elderly individuals. These areas of increased signal intensity are typically referred to as white matter hyperintensities (WMH) and can be seen on T2-weighted, proton density (PD) spin echo and Fluid Attenuated Inversion Recovery (FLAIR) images. WMH may indicate the presence of small vessel ischemic vascular disease, and are particularly common in individuals with cerebrovascular risk factors or stroke\textsuperscript{10}. However, the high prevalence of WMH observed in healthy individuals over fifty years of age suggests that they are a common age-related phenomenon\textsuperscript{11}. Unfortunately, since many segmentation procedures do not incorporate PD/T2 data, they cannot account for any change in T1-weighted contrast due to the presence of WMH.

The pathophysiological origins of WMH are diverse and include multiple cerebrovascular and neuropathological factors, ranging from so-called “incomplete” infarction\textsuperscript{12}, dilation of perivascular spaces, gliosis, demyelination, clasmotodendrosis (cytoplasmic swelling and vacuolation of astrocytes)\textsuperscript{13}, and small deep white matter microcystic and lacunar infarcts\textsuperscript{12}. Recently, harmonization standards have been recommended for research involving cognitive impairment related to vascular factors, which include suggestions for qualitative measurement of WMH\textsuperscript{14}. The current study emphasizes the need for similar guidelines in studies that attempt quantitative brain measures in individuals with dementia because of the ubiquity of white matter disease in the elderly.
Given that automated tissue segmentation techniques segment the whole brain into grey, white, and CSF volumes, the absence of an additional WMH segmentation procedure could potentially inflate volumes in some of these tissue types. Thus, depending on the signal intensities and the features of the segmentation algorithm, WMH would be allocated to the GM, WM or CSF volumes. This study quantifies this misallocation in an Alzheimer Disease (AD) dementia population with varying degrees of cerebrovascular disease to investigate this potential error. A robust T1-weighted segmentation protocol, combined with a brain regional parcellation technique, and a semi-automated lesion segmentation protocol was used to determine: a) the types of misclassification, b) the extent of misclassified tissue volumes, and, c) the brain regions most affected.

Methods

Participants: Subjects in the present study were part of the Sunnybrook Dementia Study and were recruited from the Cognitive Neurology Clinic at Sunnybrook Health Sciences Centre, a University of Toronto academic healthcare institution. Patients were excluded for this study if they had concomitant neurological disorders including clinical stroke or Parkinson’s disease, history of significant head trauma, psychotic disorder unrelated to dementia, psychoactive substance abuse, or major depression. Patients in this sub-study had the historical profile typical of AD with insidious onset of short term memory loss and gradual decline. The patients were enrolled in a longitudinal observational study using the same standardized protocol. All patients received a comprehensive clinical evaluation, including detailed medical history, neurological examination, routine
laboratory investigation and neuropsychological testing with a standardized test battery. 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, \(^{17}\) and Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition \(^{18}\) criteria for dementia. “Possible AD” diagnosis was based on the presence of sufficient subcortical cerebrovascular disease to contribute to dementia, but not enough to meet criteria for Vascular Dementia (VaD). For this study, patients were excluded if they met the National Institute for Neurological Disorders and Strokes - Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria for possible or probable VaD, which requires focal neurological signs in addition to imaging evidence of CVD. Presence of silent cortical strokes on MRI also excluded the patient.

A consensus derived rating scale developed under the auspices of the European Task Force in Age-Related White Matter Changes was used to rate WMH severity (ARWMC) \(^{19-21}\) and subclassify patients by severity of WMH (Reported \(\kappa = 0.67\); Our Group \(\kappa = 0.89\)). The ARWMC was selected because it was designed to address some of the reliability problems encountered with previous scales \(^{19}\). Details of this scale have been published elsewhere, and the ARWMC has shown promise in a study of WMH progression in AD \(^{22}\). Severity of WMH was rated on PD and T2-weighted MR images in five regions in each hemisphere: frontal, parieto-occipital, temporal, basal ganglia and infratentorial. WMH were accepted if they appeared on both PD- and T2-weighted
images and if they were at least 5mm in diameter. Severity was graded from 0 (none) to 3 (severe) based on the appearance of the WMH. A measure of global severity was derived by summing the ratings for the 5 regions.

The following criteria were applied to classify patients into Severe, Moderate, or Mild WMH subgroups. Severe WMH patients had extensive periventricular or deep white matter hyperintensities (visual rating scale score of 3 (diffuse involvement) in two areas and 2 (beginning confluence) in two other areas). Moderate WMH patients had a score of 1 (focal lesions) in more than one area or a score of two (beginning confluence) in any area. Subjects with minimal (i.e. no more than one small focal, non-lacunar hyperintensity) or no WMH were designated as having Mild WMH.

Healthy Elderly Control Group: Twenty healthy elderly controls (NC) [Mean ± SD age: 71.5±7.8] who underwent the same imaging protocol and segmentation procedure were also included in the study for additional grey matter volume comparisons. Controls were volunteers from the community.

MRI Protocols: Magnetic resonance (MR) images were acquired on a 1.5 Tesla Signa scanner (GE Medical systems) in compliance with the consensus panel imaging recommendations on Vascular Cognitive Impairment [14]. Three image sets were acquired in the same imaging session: T1-weighted (an axial 3D SPGR with 5ms TE, 35ms TR, 1 NEX, 35° flip angle, 22 x 16.5 cm FOV, 0.859 x 0.859 mm in-plane resolution, and 1.2 to 1.4mm slice thickness depending on head size), PD and a T2-weighted (interleaved axial dual-echo spin echo with TEs of 30 and 80 ms, 3s TR, 0.5 NEX, 20 x 20cm FOV, 0.781 x 0.781mm in-plane resolution, and 3mm slice thickness).
Image Analysis: Brain extraction and automated tissue segmentation was accomplished using a modified version of previously described methods\textsuperscript{1}. All images were coregistered to the T1-weighted image using the automated image registration package (AIR, v.5.2\textsuperscript{23}). The PD/T2 images were used to extract brain and sub-dural CSF, then the masked T1 was segmented using a T1-based segmentation whereby local intensity histograms are fitted to four Gaussian curves to derive cut-offs for classifying each voxel as WM, GM, or CSF. It is a robust and reliable tissue segmentation protocol optimized for elderly and AD populations (Phantom: coefficient of agreement=0.97, Scan-Rescan differences: Global < 1\% of TIC, Local < 0.15\% of TIC)\textsuperscript{1}.

Brain region parcellation was accomplished using a modified version of our previously described methods for semi-automated brain region extraction (SABRE)\textsuperscript{15}. SABRE is a highly reliable method which parcellates each individual brain into 26 brain regions proportional to individual head sizes (Inter-class Correlation range: 0.97-0.99 for individual tissue classes in each region). A set of easily identified landmarks were traced on the masked T1 images using the 3D rendering and region of interest (ROI) modules in the ANALYZE software package (Biomedical Imaging Resource, Mayo foundation, Rochester, MN, USA): the central sulcus, sylvian fissure, parieto-occipital sulcus, anterior commissure and posterior commissure. An in-house program (written in C++) combined these landmarks with the Talairach proportional grid system \textsuperscript{24} to generate individualized maps of 13 lobular regions in each hemisphere.

White matter hyperintensity segmentation was accomplished using previously described methods \textsuperscript{16}. This semi-automated procedure used an intensity cutoff based on a weighting of the PD and T2 images to define putative WMH. The output was then
manually edited by a trained operator who accepted relevant hyperintensities (based on concurrent evaluation of PD and T2 weighted images) to generate final lesion volumes (ICC range for 26 SABRE brain regions: 0.96-0.99). WMH containing cystic fluid-filled/infarcted tissue, which segmented as CSF on the T1-weighted image, were included as a sub-category of lacunar volumes.

These procedures generated two segmented images in T1-acquisition space: i) original (GM, WM, and CSF), and ii) with lesion (GM, WM, CSF, and WMH). These two images were compared globally and regionally to determine: a) the volume of misclassified tissue and b) the regions are most affected by misclassified WMH. See Figure 1 for a graphical description.

Results

Fifty-nine patients were included in the study (24 males and 35 females; mean ± SD age, 76.2 ± 6.3 yr), with a mean educational level of 13.6 ± 3.7yr. The mean ± SD MMSE score across all participants was 23.8 ± 3.8, indicating mild to moderate AD. There were more women in the groups with severe and moderate WMH ($\chi^2 = 10.1$, $p < 0.01$). Characteristics of the participants by severity of WMH are given in Table 1.

Percentage Misclassified Tissue Volumes

Percent of misclassified tissue was calculated for overall parenchyma, separated by tissue type (GM, WM), and by brain region. The mean percent misclassified tissue was greatest in the patients with severe hyperintensities (4.8%), which translates to a volume of approximately 42.2 cm$^3$. Misclassified volumes for all three severities combined were two percent (17.9 cm$^3$) of total parenchyma. Slightly greater percentages of misclassified tissue were segmented as GM, with 6.4% of total brain GM misclassified
in the group with severe hyperintensities compared to 1.2% of total brain WMH misclassified in the same group. This translates to a volume of approximately 34.7cm$^3$ and 7.5cm$^3$, respectively. Overall percentage and volumes of misclassified tissue for grey and white matter according to severity are listed in Table 2.

Data for misclassification relative to brain region is given in Table 3. Misclassified volumes were largest in the middle frontal and inferior parietal regions, particularly in the Severe WMH group where 10% of these regions were misclassified—translating to volumes of approximately 11.6cm$^3$ and 15.5cm$^3$ respectively. These same regions were also the most strongly affected when the data were collapsed across WMH group, with 4% of these regions being affected by misclassified tissue volumes.

Expressed as a proportion of total misclassified volumes, misclassified volumes were greatest in the middle and medial frontal, inferior parietal and occipital regions (see Table 3 and Figure 2). The frontal region (comprising both middle and medial frontal regions) accounted for approximately 41% misclassified tissue across all three groups, and 40% misclassified in the severe group. These percentages translate to actual volumes of approximately 7cm$^3$ and 17cm$^3$. Inferior parietal also accounted for a large percentage of misclassified tissue yielding 33% (6.4cm$^3$) of the misclassified volumes in the entire group, and 37% in the severe group (15.5cm$^3$). No hemispheric differences were seen in any of the regions. Lobular volumes of different tissue types were normalized to the supra-tentorial total intracranial capacity (ST-TIC) and expressed as a percentage. Uncorrected mean volumes expressed in cm$^3$ are provided simply to give a volumetric impression to the extent of misclassified tissue volumes.
In an additional analysis, we compared GM volumes in a group of 20 NC who underwent the same imaging protocol and segmentation procedure. As shown in Figure 3, GM volumes in the NC group were compared to our group of AD patients - with and without WMH segmentation. As expected, we found that with WMH correction, the NC group had significantly larger GM volumes than the AD patients ($F_{(3,78)}=6.05$, $P=0.001$). Bonferroni post-hoc analysis revealed greatest differences in volume when NC GM was compared to the moderate WMH ($P=0.001$) and severe WMH groups ($P=0.015$). When the groups were compared without WMH correction, a significant difference was also found ($F_{(3,78)}=5.84, P=0.001$), but post-hoc analyses revealed very different contrasts. Differences between the NC and moderate WMH remained ($P=0.03$), but inflation of the GM volume in the severe WMH group abolished the group difference ($P=1$) originally present with separate WMH segmentation. In fact, the GM mean volume of the severe WMH group exceeded that of the NC group. As shown in Figure 4, similar results were found for the Middle Frontal region ($F_{(3,78)}=16.7$, $P<0.001$) where the GM inflation in the severe WMH group was even more pronounced when compared to the normal controls ($P<0.001$).

Discussion

The results of this study suggest that clinical investigators using only a T1-weighted MRI acquisition to derive brain tissue volumes by tissue classification techniques from elderly populations should be aware of the pitfalls. In the absence of a WMH or lesion segmentation, investigators can expect up to a 5% inflation due to misclassification. In particular, T1-based segmentations can result in a 6% inflation of
GM volumes. This misclassified GM volume translates to a volume of approximately 35 cm$^3$ (for reference a standardized ping pong ball is 32.7 cm$^3$). Furthermore, the additional comparison between healthy elderly and AD patients revealed that in individuals with large volumes of WMH, GM volumes may appear to be in the normal range if not properly segmented.

WMH are a very common finding among individuals with CVD and co-existing AD. They are particularly common in individuals with cerebrovascular risk factors or stroke, but they also occur in healthy individuals over fifty years old and are increasingly prevalent with aging, suggesting they are an age-related phenomenon. One large population based study reported the prevalence of WMH to be approximately 95% in an elderly sample, with the proportion of WMH increasing with age. Similar numbers were reported in the Cardiovascular Health Study, a large (N=3301) study of community dwelling elderly, suggesting that only 4.4% of patient MRI scans were free of any abnormal signal in the white matter.

Although the current results are based on a modest sample of subjects with varying degrees of white matter disease, they are comparable to results from larger studies. The mean total cranial volume for this sample (N=59) was 1198.9 cm$^3$. These volumes are comparable to those recently reported in the Framingham Heart Study (ST-TIC=1262.8 cm$^3$, n=2200), a large community-based sample study. They also reported similar WMH volumes expressed as a percentage of total intracranial volume in subjects with white matter disease (Moderate and Severe: 3.08%, Framingham: 3.04%).

The current data indicate that regional measures of tissue atrophy in elderly populations based only on T1 segmentation should be interpreted with caution, especially...
when examining the middle frontal, medial frontal, and inferior parietal regions. In this study, up to a quarter of the frontal regions were affected by misclassified tissue volumes, and similar findings were seen in parietal regions. These regions have been previously reported to be clinically relevant in individuals with Alzheimer’s disease and with subcortical hyperintensities \[^{10}\]. Specifically, Tulberg et al. (2004) conducted a PET–study in which higher frontal and parietal WMH were associated with reduced frontal regional PET glucose metabolism and low scores on executive function tasks. Another study suggested that both the volume of WMH and the total cortical GM volumes were correlated with reduced regional cerebral glucose metabolism primarily in the dorsolateral prefrontal cortex, suggesting that WMH and GM volumes both correlate with cognitive functions usually attributed to frontal regions \[^{31}\]. The purpose of the current study was to demonstrate the need for a standardized protocol and deployment of a WMH segmentation program when planning an MRI-based volumetric study in an elderly population. Whether dealing with normal elderly controls, or patients with AD, cerebrovascular disease, or mixed diseases, clinical researchers now have a selection of automated and semi-automated lesion segmentation protocols to choose from to minimize the problem of misclassified tissue volumes in individuals with WMH \[^{32,33,34,6,11,35}\]. Novel unified multispectral segmentation algorithms may better solve the problem of misallocated tissue due to WMH, however, to our knowledge there are currently no such programs that include a WMH segmentation \[^{36,37,38}\]. Currently, to implement quantitative measures of WMH, MRI scanning protocols must include additional T2-weighted, PD and/or FLAIR images. In compliance with the consensus imaging recommendations for
Vascular Cognitive Impairment, we suggest similar standards for AD studies due to the high prevalence of subcortical ischemic vascular disease\textsuperscript{14}.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mild WMH</th>
<th>Moderate WMH</th>
<th>Severe WMH</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Subjects</td>
<td>19</td>
<td>22</td>
<td>18</td>
<td>ns</td>
</tr>
<tr>
<td>Men/women</td>
<td>13/6</td>
<td>6/16</td>
<td>5/13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age, y</td>
<td>74.8 (5.8)</td>
<td>75.6 (6.3)</td>
<td>78.2 (6.6)</td>
<td>ns</td>
</tr>
<tr>
<td>MMSE</td>
<td>24.3 (3.7)</td>
<td>23.6 (3.7)</td>
<td>24.5 (4.0)</td>
<td>ns</td>
</tr>
<tr>
<td>YOE</td>
<td>14.1 (4.4)</td>
<td>13.3 (3.6)</td>
<td>13.0 (3.3)</td>
<td>ns</td>
</tr>
<tr>
<td>ARWMC score</td>
<td>1.0 (0.9)</td>
<td>8.7 (3.7)</td>
<td>16.7 (3.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$\chi^2$ and One-way ANOVA group comparisons. Numbers are mean (SD).
YOE = Years of Education; MMSE = Mini-Mental State Examination\textsuperscript{59}
ARWMC = Age-related White Matter Changes Scale\textsuperscript{40}
Table 2. Total Misclassified Tissue by Group

<table>
<thead>
<tr>
<th></th>
<th>Total (N=59)</th>
<th>Severe WMH (n=18)</th>
<th>Moderate WMH (n=22)</th>
<th>Mild WMH (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percent (%)</strong></td>
<td>2.04</td>
<td>4.83</td>
<td>1.33</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Volume (cm$^3$)</strong></td>
<td>17.9 (0.2-86.5)</td>
<td>42.2 (18.1-86.5)</td>
<td>11.4 (0.2-24.3)</td>
<td>2.5 (0.2-6.3)</td>
</tr>
<tr>
<td><strong>Grey Matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Percent (%)</strong></td>
<td>2.73</td>
<td>6.41</td>
<td>1.68</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Volume (cm$^3$)</strong></td>
<td>14.2 (0.1-78.0)</td>
<td>34.7 (13.0-78.0)</td>
<td>8.4 (0.2-18.5)</td>
<td>1.6 (0.1-4.6)</td>
</tr>
<tr>
<td><strong>White Matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Percent (%)</strong></td>
<td>1.03</td>
<td>1.16</td>
<td>0.42</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Volume (cm$^3$)</strong></td>
<td>3.7 (0.04-17.2)</td>
<td>7.5 (3.3-17.2)</td>
<td>3.0 (0.04-7.6)</td>
<td>0.9 (0.05-2.6)</td>
</tr>
</tbody>
</table>

Values in parentheses indicate range. Percent MRI measures are expressed as mean percent of supra-tentorial total intracranial capacity (ST-TIC) (Total =879.1cm$^3$; Severe=872.6cm$^3$; Moderate=858.8cm$^3$; Mild=908.7cm$^3$).
Table 3. Percentage of Misclassified Volumes in each SABRE Brain Region

<table>
<thead>
<tr>
<th>Regions</th>
<th>Total (N=59)</th>
<th>Severe SH (n=18)</th>
<th>Moderate SH (n=22)</th>
<th>Mild SH (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent (%)</td>
<td>Volume (cm³)</td>
<td>Percent (%)</td>
<td>Volume (cm³)</td>
</tr>
<tr>
<td>Sup Frontal</td>
<td>1.23</td>
<td>0.6 (0-5.1)</td>
<td>1.7 (0.02-5.1)</td>
<td>0.2 (0-1.2)</td>
</tr>
<tr>
<td>Mid Frontal</td>
<td>4.497</td>
<td>5.1 (0-21.0)</td>
<td>11.6 (2.7-21.0)</td>
<td>3.5 (0-6.7)</td>
</tr>
<tr>
<td>Inf Frontal</td>
<td>0.338</td>
<td>0.1 (0-0.9)</td>
<td>0.2 (0-0.9)</td>
<td>0.04 (0-0.2)</td>
</tr>
<tr>
<td>Med Inf Frontal</td>
<td>0.492</td>
<td>0.1 (0-0.8)</td>
<td>0.2 (0-0.8)</td>
<td>0.04 (0-0.2)</td>
</tr>
<tr>
<td>Med Sup Frontal</td>
<td>0.845</td>
<td>0.4 (0-3.4)</td>
<td>1.0 (0.05-3.4)</td>
<td>0.1 (0-0.5)</td>
</tr>
<tr>
<td>Med Mid Frontal</td>
<td>2.390</td>
<td>1.1 (0-4.8)</td>
<td>2.0 (0.5-4.8)</td>
<td>1.0 (0-2.7)</td>
</tr>
<tr>
<td>Sup Parietal</td>
<td>1.030</td>
<td>6.4 (0-13.5)</td>
<td>2.7 (0.03-13.5)</td>
<td>0.3 (0-1.1)</td>
</tr>
<tr>
<td>Inf Parietal</td>
<td>4.376</td>
<td>1.4 (0-13.2)</td>
<td>3.3 (0.02-13.2)</td>
<td>0.8 (0.1-2.2)</td>
</tr>
<tr>
<td>Occipital</td>
<td>1.434</td>
<td>1.4 (0-13.2)</td>
<td>3.3 (0.02-13.2)</td>
<td>0.8 (0.1-2.2)</td>
</tr>
<tr>
<td>Ant Temporal</td>
<td>0.845</td>
<td>1.0 (0-1.7)</td>
<td>0.2 (0-1.7)</td>
<td>0.01 (0-0.05)</td>
</tr>
<tr>
<td>Post Temporal</td>
<td>0.338</td>
<td>6.4 (0-13.5)</td>
<td>2.7 (0.03-13.5)</td>
<td>0.3 (0-1.1)</td>
</tr>
<tr>
<td>Ant BG/Thal</td>
<td>0.782</td>
<td>1.6 (0-6.1)</td>
<td>3.5 (0.6-6.1)</td>
<td>1.3 (0-3.9)</td>
</tr>
<tr>
<td>Post BG/Thal</td>
<td>0.212</td>
<td>0.4 (0-1.7)</td>
<td>0.2 (0-1.7)</td>
<td>0.01 (0-0.01)</td>
</tr>
</tbody>
</table>

Values in parentheses indicate range.
Percent MRI measures are expressed as mean percent of total regional capacity (TRC).
Figure 1 – A) 3-dimensional, surface CSF eroded, T1-weighted translucent brain with misclassified volumes shown in red. B) SABRE parcellated brain with different colors denoting different brain regions. C) SABRE parcellated translucent brain with colors denoting brain regions, misclassified volumes shown in red. i-vi) Representative axial slice illustrating the images and steps required to generate A-C: i) T1-weighted image, ii) T1-segmentation, iii) proton density, iv) proton density with lesion overlay, v) T1-segmentation with lesion overlay, vi) SABRE parcellated brain with misclassified volumes overlayed.

Figure 2 – Data expressed as a percentage of whole brain misclassified volumes across all groups for 13 SABRE brain regions.

Figure 3 - Mean GM volumes of AD patients with and without WMH segmentation as compared to NC. Volumes corrected for head size using the supra-tentorial total intracranial capacity (ST-TIC). Figure shows that without WMH correction, inflation of GM volumes in the severe WMH-AD patients approach normal.

Figure 4 - Mean GM volumes of AD patients with and without WMH segmentation as compared to NC in the middle frontal region. Volumes corrected for total regional capacity (TRC). Figure shows that without WMH correction, inflation of GM volumes in the AD patients approach or exceed normal.
Middle Frontal Grey Matter Volumes With and Without WMH Correction: Comparison with Normal Control
Reference List


