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FACULTY OF MEDICINE

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Perfusion SPECT Imaging in Dementia Diagnosis: Current Utility and Clinical Validity

by

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ABSTRACT

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Despite the extensive development of image analysis techniques over recent years, visual reading of regional cerebral blood flow (rCBF) single-photon emission computed tomography (SPECT) imaging for dementia diagnosis is common and reporting standards vary widely. Newer quantitative methods of analysis can provide additional diagnostic power for dementia over visual assessment, however these are poorly validated for clinical practice, with prior research rarely using clinically applicable samples or analyses that consider the individual. This dissertation presents a series of translational studies using naturalistic samples that assess the clinical utility of rCBF SPECT imaging using updated analysis methods to aid diagnosis, prognosis and clinical decision making.

The ability of perfusion SPECT imaging using quantitative methods of analysis to predict diagnosis and future decline was validated using independent biomarkers of pathology and longitudinal functional decline. The use of posterior cingulate preservation of perfusion combined with occipital hypoperfusion had high specificity (98%) and positive prediction (11.1) for abnormal dopamine transporter SPECT (DaTSCAN) imaging, and therefore dementia with Lewy body detection. The angular gyrus and precuneus were found to be the most salient regions for prediction of cerebrospinal fluid biomarker status indicative of Alzheimer's disease (AD), and in combination with other regions, could predict those at high risk of AD from low risk individuals (accuracy 74%). Patients with abnormal imaging on perfusion SPECT

showed worse survival over 18 months follow-up on Kaplan-Meier curve analysis and a faster rate of decline than those with normal imaging, highlighting the ability of rCBF SPECT to detect individuals with neurodegenerative disease.

To determine whether additional information beyond diagnosis could be gained from perfusion SPECT imaging, associations between patient imaging and caregiver burden were completed. Caregiver burden was strongly related to the extent of frontal or right-predominant parietal or temporal lobe dysfunction, suggesting that regional abnormality on perfusion SPECT can facilitate identification of individuals who are likely to create a high burden on caregivers.

Finally, the utility of perfusion SPECT imaging to influence diagnosis and diagnostic confidence was assessed using a two part questionnaire completed by referring clinicians. Diagnosis changed after imaging in 44% of cases and confidence in diagnosis was significantly improved in those who were less than 50% confident prior to imaging. When asked directly, 96% of clinicians stated that the imaging contributed to the diagnostic process.

The findings from these studies provide evidence for the clinical use of quantitative methods of analysis with perfusion SPECT imaging in dementia diagnosis, prognosis and identification of care needs, and show the technique is valued by clinicians, influencing diagnosis and diagnostic confidence.

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DECLARATION OF AUTHORSHIP

I, Angus Michael James Prosser, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Perfusion SPECT imaging in dementia diagnosis: current utility and clinical validity

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

Journal articles:

Prosser AMJ, Tossici-Bolt L, Kipps CM. Occipital lobe and posterior cingulate perfusion in the prediction of dementia with Lewy body pathology in a clinical sample. Nucl Med Commun. 2017;38(12):1029-35

Prosser AMJ, Spreadbury JH, Tossici-Bolt L, Kipps CM. Imaging Care Requirements: Use of Functional Neuroimaging to Predict Dementia Caregiver Burden. Dement Geriatr Cogn Dis Extra. 2018;8:180-9

Abstracts:

Prosser AMJ, Spreadbury JH, Tossici-Bolt L, Kipps CM. Imaging care requirements: Use of functional HMPAO-SPECT scanning to predict caregiver burden, Alzheimer's & Dementia. 2017;13(7):P494

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Signed:.....

Date: .....

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This thesis is dedicated to Dr David G. Hawksworth.

Definitions and Abbreviations

AD – Alzheimer's disease

CBI – Cambridge Behavioural Inventory

CBD – Corticobasal degeneration

CDR – Clinical Dementia Rating

CSF – Cerebrospinal fluid

CT – Computed tomography

DaTSCAN – [123I]-2b-carbomethoxy-3b-(4-iodophenyl)-N-(3-fluoropropyl)nortropane (123I-FP-CIT) SPECT

DLB – Dementia with Lewy bodies

FAQ – Functional Activities Questionnaire

FTD – Frontotemporal dementia

HMPAO – Hexamethylpropyleneamine oxime

MCI – Mild cognitive impairment

MRI – Magnetic resonance imaging

PET – Positron emission tomography

PPA – Primary progressive aphasia

PSP – Progressive supranuclear palsy

rCBF – Regional cerebral blood flow

SPECT – Single photon emission computed tomography

VaD – Vascular dementia

ZBI – Zarit Burden Interview

Chapter 1: Literature review and introduction

1.1 Introduction

Dementia is an umbrella term for a group of neurodegenerative diseases that cause cognitive and/or behavioural impairment that affects an individual's daily living. The most common causes of dementia are Alzheimer's disease (AD), vascular dementia (VaD), dementia with Lewy bodies (DLB) and frontotemporal dementia (FTD) (1). These diseases all progressively worsen over time with memory, concentration and attention, orientation, language and executive dysfunction commonly seen among other symptoms.

It has been estimated that there are over 800,000 people with dementia in the UK, with this number predicted to rise to over 1 million by 2025 and over 2 million by 2051 (2). In 2013 care for those with dementia was estimated to cost approximately £26 billion a year, with the global cost of care exceeding $600 billion (3). Early detection of dementia and timely diagnosis allows patients to utilise care services and has been shown to improve patient and carer clinical outcomes including quality of life and delayed institutionalisation (4–8). Furthermore, accurate prognostic information and identification of individuals at highest risk of decline could allow early signposting of patients to appropriate resources. Diagnosis and prognosis in dementia can however be challenging, with clinical methods alone resulting in a significant diagnostic gap. Dementia diagnosis can take more than a year and a large proportion of those with dementia in the UK remain undiagnosed despite substantial efforts to increase diagnosis rates over recent years (9,10).

Improving dementia diagnosis rates has been a main focus in multiple national and international policies and reports on dementia (11–14). These reports have highlighted the need for measures or tools that can detect underlying dementia pathology, monitor disease progression and predict decline in the individual (biomarkers). Ideally, these biomarkers should be specific to a single dementia disease, reproducible, objective and directly reflect the dementia pathological process. For a biomarker to be deemed useful in dementia, it should be shown to have a greater than 80% sensitivity (the ability of the

criteria to detect the disease when it is present) and specificity (the ability of the criteria to classify a patient as normal when the disease is not present) when validated in neuropathologically confirmed cases of dementia and controls (15). Currently, no dementia biomarkers satisfy these criteria for the ideal biomarker, nor has one biomarker reached universal acceptance. Neuroimaging is however at the forefront of biomarker use in dementia, with both structural and functional imaging able to detect pathology before the clinical onset of dementia, accelerate diagnosis, monitor disease progression, and offer prognostic information to optimise management.

The neuroimaging technologies most commonly used in clinical practice for dementia diagnosis are computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT).

1.1.1 Neuroimaging in dementia

Changes in the structure and function of the brain have been shown to occur before presentation of dementia symptoms (16,17), allowing brain imaging techniques to provide further information when detailed history taking and dementia screening tests are unable to give sufficient information for confident diagnosis (18). Neuroimaging techniques allow visualisation of disease pathology such as brain atrophy, functional deficit and abnormal protein aggregations in patients *in vivo*. Due to characteristic patterns of structural or functional deficits differing between dementia subtypes, neuroimaging patterns can be used as biomarkers for different dementia syndromes. These patterns can be used to either identify or exclude suspected dementia subtypes from other pathologies with similar symptoms. There are a number of papers which describe common atrophy and functional patterns in each dementia subtype (19–26), with neuroimaging patterns for the most common dementias outlined in Table 1-1. For example, in Alzheimer's disease a typical pattern of global atrophy with pronounced atrophy of the medial temporal lobes, reduced activity in the parietal cortex and increased amyloid-beta deposits in the brain is commonly seen. Frontotemporal dementia, dementia with Lewy bodies and vascular dementia also show characteristic imaging patterns which can be utilised for differentiation between pathologies.

1.1.1.1 Structural brain imaging

Structural imaging is recommended in all patients with suspected dementia and can provide detailed anatomical information on the structure of the brain (18). It is used for exclusion of non-dementia cerebral pathologies and assessment of vascular damage, white matter changes and cerebral atrophy in dementia, with atrophy assumed to be a surrogate marker of neurodegenerative change. MRI is commonly preferred for structural neuroimaging over CT, as it does not use ionising radiation and has high grey to white matter contrast. MRI is usually visually assessed in clinical situations, however more sophisticated quantitative measurements of atrophy are available and used in research. In cases where the structural imaging is inconclusive, functional brain imaging using radionuclides is recommended (18).

1.1.1.2 Functional brain imaging

Nuclear imaging using radioactive tracers has been shown to be an effective tool in aiding dementia diagnosis with SPECT and PET able to provide information on perfusion, metabolism, neurotransmitter availability and abnormal protein deposits in the brain (23,24). Perfusion (SPECT) and metabolic (PET) imaging both give an indirect measurement of brain function using radioactive tracers, with functional deficits indicated by reduced blood flow (hypoperfusion) or metabolism (hypometabolism) often seen before structural brain changes in dementia (27). Tracers are also available for use with SPECT imaging to measure dopaminergic activity in the striatum (123I-FP-CIT DaTSCAN imaging), with dopaminergic deficits seen in Parkinson's disease, Parkinsonian disorders and dementia with Lewy bodies (28,29).

Despite the development of new tracers to track disease pathways through abnormal protein deposits, such as those that bind to amyloid-beta, tau and alpha-synuclein accumulations, perfusion SPECT imaging remains one of the most frequently used functional imaging techniques to aid dementia diagnosis in Europe and Japan (30). Perfusion SPECT is a longstanding technology that allows visualisation of regional cerebral blood flow (rCBF), an indirect measure of neural activity at rest, using a radioactive tracer (31). Tracer uptake in brain tissue is proportional to blood flow, allowing visualisation of perfusion and corresponding brain function.

1.2 Perfusion SPECT imaging in dementia procedure and analysis

1.2.1 Procedure

Perfusion SPECT imaging uses gamma cameras to detect gamma rays emitted by a radioactive tracer as it decays. The three most common types of perfusion brain SPECT tracers used are (99m)Technetium-hexamethylpropyleneamine oxime (99mTc-HMPAO), (99m)Technetium-ethyl-cysteinate dimer (99mTc-ECD), and (123)Iodine N-isopropyl-p-iodoamphetamine (123I-IMP). The use of 99mTc-HMPAO and 99mTc-ECD are preferred to 123I-IMP as they are more readily available. Prior to scanning, the tracer is injected into the patient and removed from the blood by brain tissue in a manner proportional to the blood flow at the time of injection. The individual being investigated is placed in a SPECT camera shortly after tracer injection and asked to stay still for approximately 30 to 60 minutes, where single gamma ray emissions from the tracer are detected at multiple angles around the patient. The emissions detected are reconstructed into multiple planar images, which are then combined to produce a three-dimensional image. Reconstruction methods vary, with filtered back projection or ordered subset maximisation commonly used. Sophisticated scatter and attenuation correction methods may also be used to improve resolution and sensitivity of images. Recommended procedure guidelines for brain perfusion SPECT are described in detail by Juni et al (2009) and Kapucu et al (2009) (32,33).

1.2.2 Image analysis and clinical reporting

Accuracy of perfusion SPECT in dementia identification varies widely depending on the analysis technique used to evaluate the image. Hypoperfusion patterns for dementia diagnosis are usually interpreted by visual assessment of the reconstructed image (qualitative) or a visual read of a quantitative analysis that has been applied to the image (semi-quantitative). More recently, advanced computational methods of analysis that are purely quantitative are also available, but are yet to enter general clinical use due to the expertise needed to complete the analysis.

1.2.2.1 Visual assessment

Perfusion brain SPECT imaging is most commonly interpreted by an expert reader through qualitative visual assessment. This is completed by visually interpreting a reconstructed image using axial slices or three-dimensional renderings. A continuous colour scale is added to the image to represent cerebral perfusion, with a heat scale often used (Figure 1-1). The image interpreter (reporter) looks for areas with reduced perfusion in specific regions using the colour scale. As some dementia patterns are predominantly one sided, the reporter can also compare perfusion between hemispheres to look for areas of hypoperfusion. Reporters may also evaluate the SPECT image in the context of patient structural imaging (MRI / CT), with particular attention to be paid to areas of morphological abnormality and atrophy (33). Reporting images in a purely qualitative way requires substantial expertise and experience, with changes on imaging often subtle in early disease. Guidelines for SPECT interpretation recommend that the reporter is familiar with a reference database created by the Society of Nuclear Medicine (SNM) Brain Imaging Council to aid visual assessment of images (32). Visual assessment of dementia patterns is the oldest method of perfusion SPECT interpretation and patterns of hypoperfusion have been defined for all dementia subtypes.

Despite guidelines on processing and interpretation of visual images, inter-rater reliability is low, particularly with inexperienced reporters (34,35). Changes in perfusion in dementia can often be minimal and the relatively low resolution inherent in SPECT imaging combined with the lack of a direct comparison against a normal group makes identification of specific regions of hypoperfusion difficult. The use of semi-quantitative analysis, where a computerised analysis method is visually assessed for patterns of hypoperfusion indicative of dementia disease, can provide improved detection of perfusion deficits over visual assessment alone (22,34–37). This improved sensitivity for dementia is shown in a review by Frisoni et al (2013), where visual and semi-quantitative perfusion image analysis studies were compared (22).

1.2.2.2 Semi-quantitative analysis

Semi-quantitative analysis produces an output that identifies regions of hypoperfusion (or hyperperfusion) in a disease group or individual in comparison to another group or individual (Figure 1-1). This output is then visually interpreted by a trained reporter for patterns suggestive of dementia disease. Semi-quantitative analyses include statistical parametric mapping (SPM) and three-dimensional stereotactic surface projection (3-D SSP). Other methods of analysis are available, however will not be described here due to their limited use in current clinical practice. Visual assessment of SPM and 3-D SSP output requires expertise to identify perfusion deficit patterns of dementia, however reporters with limited training can interpret the images with increased accuracy and inter-rater reliability when compared to pure qualitative visual assessment (34,35).

1.2.2.2.1 Statistical Parametric Mapping (SPM) and three-dimensional stereotactic surface projections (3-D SSP)

Statistical parametric mapping (SPM) is a free to use automated voxel-based imaging approach that runs through MATLAB software (38). SPM builds on the methods of subtraction images, where one image is subtracted from another to leave only that which is different between the two images. SPM uses voxel-based morphometry (VBM), which assesses the local brain function after macroscopic changes in morphological differences are discounted. As VBM assumes that data from a particular voxel on each image originates from the same part of the brain, it requires several processing steps to prevent variance in the images that could introduce artificial differences or mask real differences between groups. Processing includes translating, transforming and warping all images into a common stereotactic space (spatial normalisation) and averaging the signal over a range of neighbouring voxels at each voxel (smoothing) before statistical comparisons are made. Comparisons of the concentration of rCBF in SPECT (metabolism in PET or grey matter in MRI or CT) between two groups of subjects voxel by voxel in a mass-univariate approach are then completed. The Student's *T* and *F* tests are often used when evaluating groups in both research and clinical settings with either a group against group or individual against group analysis. The resulting statistical parameters are then used to produce a colour-graded image showing group

differences; the statistical parametric map. This map is equivalent to a t-value or z-score map, which can then be visually assessed.

Three-dimensional stereotactic surface projections are another popular method of visualising differences between two groups or an individual and a group (39). 3-D SSP software analyses images on a pixel-by-pixel basis, with the outer and medial surfaces of the brain of the two groups compared. This is different to VBM, where all voxels in the cortex are compared. A pre-processing procedure similar to that carried out for SPM is first completed before image analysis. As with SPM, the original images are all aligned into a standard stereotactic space using translations, transformations and linear and non-linear warping. For analysis, mean and standard deviation values for every pixel in each group are calculated and z-scores for each pixel-by-pixel comparison are created. Results from 3-D SSP can be displayed either as a diagnostic index, where the positive z-scores (hypoperfusion in the disease group) are averaged globally or for specific regions of interest, or as a 3-D surface map (the 3-D SSP). The 3-D map is created by displaying the z-scores on a standard contour of the brain.

Both SPM and 3-D SSP analysis can be completed on specific regions of the brain, rather than across the whole cerebrum. This is called region of interest (ROI) analysis and has a number of benefits over global analysis. ROI analysis reduces the number of voxel by voxel (or pixel-by-pixel) analyses completed in mass-univariate analysis, reducing the likelihood of false positive data and the need for highly stringent multiple comparison corrections. In addition, pre-processing smoothing methods can often make definition of precise anatomical boundaries difficult. ROI analysis allows the researcher to define a specific region for viewing perfusion deficits. Definition of ROIs can also be used to extract perfusion values from pre-specified regions. These values can then be used to provide more information than SPM or 3-D SSP global analysis alone and allows other statistical analysis such as correlations between regions, or with neuropsychological assessments for instance, to be completed.

1.2.2.2.2 Individual pattern analysis

Semi-quantitative and quantitative methods can be used to compare two groups of disease patients or controls to provide areas of combined perfusion

differences at a specified threshold. This group by group analysis provides regions of clear perfusion differences that have been validated in multiple dementia subtypes. Although group difference maps give information on the general pattern of perfusion deficits for a disease group, they reflect an accumulation of all the individual patterns of perfusion loss and therefore may not be representative of perfusion patterns seen in individuals. Individuals are unlikely to present with all regional deficits seen in group analysis, particularly in early disease, and smaller changes in perfusion that are seen in some individuals, but not all, may be missed. Group difference analysis is also of little use to clinicians who require information on perfusion deficits of an individual. Clinical analysis techniques that focus on individual perfusion patterns are available, as well as research analysis techniques that try to overcome the issues of group-by-group analysis.

In SPM and 3-D SSP, analysis of an individual against a normal database can be completed using a one-sample, or two-sample unpaired t-test. For a one-sample t-test, contrast images are created by subtracting each control image from an individual patient image. These subtraction images are then entered into a one-sample t-test. An unpaired two-sample t-test using a "one versus many" approach provides similar information, such as that described by Kemp et al (2005) (36). The resulting maps can be used by clinicians in combination with knowledge of group analysis patterns to aid diagnosis and prognosis in cases where there is diagnostic uncertainty.

1.2.2.2.3 Limitations of mass-univariate approaches

Statistical analysis using SPM and 3-D SSP does however have several limitations, the first being an issue of multiple comparisons. SPECT images using a matrix of 256x256 are constructed of more than 65,000 voxels, and therefore at a threshold of $p < .05$ around 3250 false positives are expected. At a more conservative threshold of $p < .001$ around 65 false positive results are still expected to be found. A tongue-in-cheek study using MRI and mass univariate methods to image a dead salmon highlighted this issue, with apparent regions of activity found in the fish's brain cavity (40). When an individual against group analysis is completed, group level parametric assumptions are no longer met, which may lead to a further increase in false positive rates (41,42). Multiple comparison corrections such as familywise error rate (e.g. Bonferroni) can also be used, but are often too conservative for

mass univariate approaches, masking real differences in study populations. The multiple comparison problem can be minimised by image spatial smoothing, the use of region of interest (ROI) analysis and the implementation of a voxel cluster threshold (extent threshold), but not prevented. Secondly, mass-univariate approaches such as SPM and 3-D SSP also assume that the brain has unchanging correlations between different parts of the brain, modelling the maps as a stationary process. Hypothesis testing is based on group-level contrasts, with the assumption that different brain locations are independent from one another. This is however not the case, with functional anatomical correlations indicating complex interconnections of the brain. The mass-univariate approach therefore may not acknowledge correlations between contrasts in different brain regions. Multivariate analysis techniques approach the brain as a connectome, with correlations between different brain regions assumed.

1.2.2.3 Novel and developing analysis and reporting techniques

Purely quantitative methods of analysis are becoming more commonplace in research, however these are still rare in clinical use. With the use of multiple modalities now recommended in a single dementia diagnosis and global technological advances in computing, there is now the potential to combine functional, structural and other modalities into one reporting system (Figure 1-1). In addition, there has been an increased focus on the use of automated means of detecting dementia. These systems include the use of principal component analysis, support vector machines and neural networks; algorithms that automatically detect the best way to separate two or more groups of data. This can then been used to separate dementia patients from controls or even separate two different dementia subtypes (43,44). These methods include multivariate and multimodal analysis, and provide information about brain networks and relationships between separate modalities respectively without the need for *a priori* hypotheses and ROI placement.

Multivariate analysis allows detection of diffuse patterns that could not be detected in univariate models of analysis such as voxel based morphometry. Multivariate analysis consider correlations between contrasts and voxels, which can generally reduce variability and increase statistical power (38). Quantitative analysis by support vector machines (SVMs) and similar systems can also

analyse data from different modalities that are normally analysed individually, such as MRI, SPECT and CSF biomarkers, and detect patterns between them. SVMs could be used to classify an individual as either disease or normal, and give probabilities on how well the patient fits into either group.

Multivariate and multimodal techniques are not commonly used in clinical practice due to lack of evidence of real-world clinical usefulness and the need for technical expertise for their use. Although they have been shown to be highly effective at detecting dementias in controlled disease samples (44,45), their ability to aid diagnosis in an undefined clinical sample of patients is unknown, and translational studies are required.

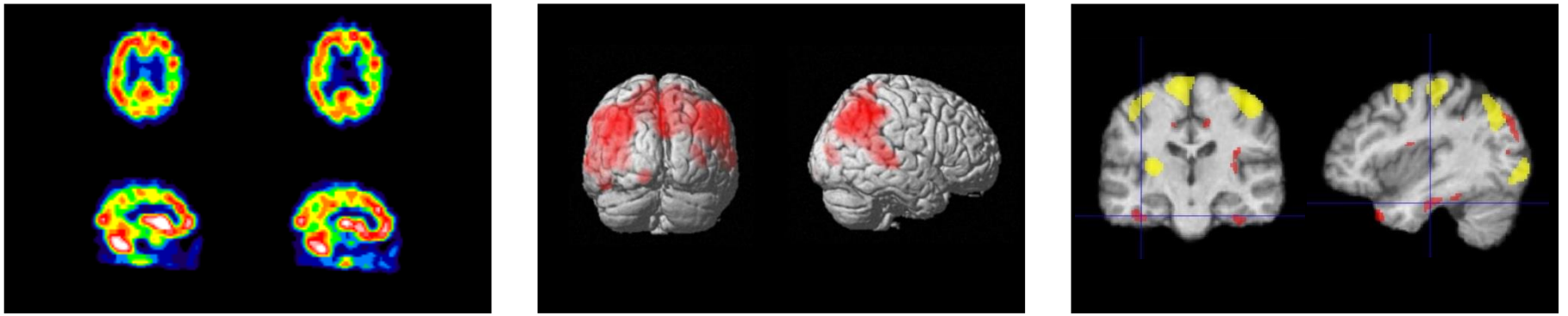


Figure 1-1 Visual, semi-quantitative and multimodal analysis of perfusion SPECT images

Examples of visual (left), statistical parametric mapping (middle) and multimodal voxel based analysis (right) for interpretation of perfusion SPECT images. Visual images show reconstructed axial slices, with SPM analysis showing areas of hypoperfusion in red displayed on a standard structural template to allow easier localisation of hypoperfused regions. Multimodal imaging example shows areas of hypoperfusion (yellow) and atrophy (red) after voxel based morphometry on both SPECT and structural MRI imaging in the same patient.

1.3 Perfusion SPECT imaging patterns in dementia diagnosis

Perfusion SPECT using visual, semi-quantitative and quantitative analysis shows typical patterns of hypoperfusion that can indicate specific dementia disease subtype. Typical structural imaging (by MRI and CT), functional imaging (by perfusion SPECT / MRI, or metabolic PET) and molecular imaging (by amyloid PET and 123I-FP-CIT SPECT) patterns for AD, FTD, DLB, VaD, corticobasal degeneration, progressive supranuclear palsy and posterior cortical atrophy are described in Table 1-1. As 95% of the dementia population is made up of AD, FTD, DLB and VaD, only these subtypes and current knowledge on SPECT patterns will be discussed in detail (1,2).

Table 1-1 Common neuroimaging patterns for dementia disease subtypes

Patterns on functional perfusion imaging correlate with those seen on metabolic imaging. Combinations of dementia disease types can occur in individuals, blurring pattern boundaries.

Disease	Structural imaging	Functional imaging	Molecular imaging
Alzheimer's Disease	Global non-specific atrophy of cerebral cortex and white matter tracts, with ventricular enlargement and specific volume loss in the medial temporal lobe	Bilateral temporoparietal hypoperfusion particularly in the precuneus, posterior cingulate and angular gyrus	Positive amyloid PET
Vascular Dementia	Non-specific cerebral cortex atrophy, white matter hyperintensities	'Patchy' pattern of hypoperfusion across the brain	Negative amyloid PET
Dementia with Lewy Bodies	Global non-specific atrophy with relative preservation of the medial temporal lobe	Bilateral temporoparietal and occipital hypoperfusion. Posterior cingulate gyrus function is maintained	Loss of dopaminergic function as assessed by DaTSCAN imaging. Amyloid PET is variable.
Behavioural variant frontotemporal dementia	Atrophy in the frontal and/or temporal lobes	Hypoperfusion in the frontal and/or temporal lobes	Negative amyloid PET

Table 1-1 Common neuroimaging patterns for dementia disease subtypes (cont.)

Disease	Structural imaging	Functional imaging	Molecular imaging
Semantic dementia	Left hemisphere predominant atrophy of the anterior temporal lobe	Anterior temporal lobe hypoperfusion	Negative amyloid PET
Progressive non-fluent aphasia	Left hemisphere predominant atrophy of the inferior frontal lobe	Left hemisphere predominant posterior fronto-insular hypoperfusion	Negative amyloid PET
Logopenic aphasia	Left hemisphere predominant posterior perisylvian or parietal atrophy	Left hemisphere predominant perisylvian hypoperfusion	Positive amyloid PET
Corticobasal degeneration	Asymmetric cerebral cortex atrophy of the supplementary motor area, insula and premotor cortex	Asymmetric hypoperfusion in the posterior frontal, parietal, basal ganglia and thalamus regions	Loss of dopaminergic function as assessed by DaTSCAN imaging

Table 1-1 Common neuroimaging patterns for dementia disease subtypes (cont. 2)

Disease	Structural imaging	Functional imaging	Molecular imaging
Progressive supranuclear palsy	Atrophy of the midbrain, frontal lobes, supplementary motor area, caudate nucleus and putamen	Frontal, anterior parietal, caudate nucleus and thalamus hypoperfusion	Loss of dopaminergic function as assessed by DaTSCAN imaging.
Posterior cortical atrophy	Cortical atrophy of the parietal and occipital lobes	Posterior hypoperfusion	Positive amyloid PET

1.3.1 Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia, estimated to be responsible for around 60% of all dementia's seen in the UK (1,2). It is characterised by prevailing and worsening deficits in memory, language and/or behavioural disturbance; impairment in executive function and loss of the ability to perform complex (and later simple) tasks. The typical AD brain at autopsy shows global atrophy with disproportionate volume loss in medial temporal lobe structures. The neuronal loss seen in Alzheimer's disease is believed to be driven by pathological concentrations of amyloid-β42 (Aβ42) and hyperphosphorylated tau in the brain. Increases in the concentrations of these proteins leads to the formation of neuritic plaques and neurofibrillary tangles, hallmarks of Alzheimer's disease pathology (46).

Symptoms and pathology of Alzheimer's disease are well described in the literature and form the basis of the clinical criteria for diagnosis of AD. Several criteria are available for AD diagnosis including the National Institute of Neurological disorders and Stroke – Alzheimer Disease and Related Disorders (NINCDS-ADRDA) (47), the National Institute on Aging (NIA) criteria for AD and mild cognitive impairment (48,49) and the National Institute for Health and Clinical Excellence (18). Criteria recommend the use of both structural and functional imaging when there is diagnostic uncertainty after clinical assessments.

Due to the prevalence of AD, the vast majority of neuroimaging research on dementia is focussed on the AD subtype and typical neuroimaging patterns are well established. Structural changes using MRI and CT reflect that seen at autopsy, with hippocampal and medial temporal lobe (MTL) atrophy giving good accuracy for AD (50–52).

1.3.1.1 Alzheimer's disease perfusion SPECT pattern in diagnosis

The pattern of functional deficits in Alzheimer's disease differs from the regions of atrophy seen in structural imaging, with bilateral hypoperfusion in the temporoparietal region the best validated marker for disease. This includes the inferior parietal, posterior temporal and angular gyrus regions (Figure 1-2). Temporoparietal hypoperfusion was first suggested as a biomarker for AD by Ingvar et al in 1975, who used ^{133}Xe and 2-D imaging to show rCBF deficits in

AD (53). This has been supported by numerous ^{123}I and ^{99m}Tc SPECT tracer studies; the majority using visual analysis techniques (34,54–59) or older region of interest analysis (58,60–66). Studies using semi-quantitative voxel based analysis methods have shown similar results with temporoparietal deficits in AD (34,67–74). Comprehensive meta-analysis papers on the specificity and sensitivity of perfusion SPECT imaging in dementia show values ranging from approximately 71% to 80% and 72% to 89% respectively, depending on image analysis and disease samples investigated (22,34,75,76). Clinicopathological studies using SPECT imaging and neuropsychological or cognitive tests show that reduced temporoparietal region perfusion correlates with a loss of multidomain cognitive function when compared to controls, particularly memory and visuospatial deficits (77,78).

In addition to temporoparietal hypoperfusion, perfusion deficits in the medial parietal gyri such as the precuneus have been implicated in the disease (36,79–82). The posterior cingulate regions have also been shown to be hypoperfused in AD patients when compared to controls (35,67,68,82–90). Posterior cingulate regional atrophy has been shown to occur before symptom onset, which may explain the loss of function seen in this region on perfusion SPECT (91). As the temporoparietal and precuneus regions rarely show disproportionate levels of atrophy in AD in comparison to the rest of the cerebrum, it is believed that the loss of function may be related to disconnection from efferent fibres that connect the medial temporal lobe and these cortices (67).

Despite structural changes in the hippocampus being an accurate predictor of AD, the utility of hypoperfusion in the medial temporal lobe as a biomarker is less clear. Many studies have shown significant reductions in MTL or hippocampal perfusion in AD and progressive MCI when compared to controls (61,74,78,92–100). A study correlating Braak histopathological staging with SPECT imaging found the anterior medial temporal lobe, along with posterior cingulate changes, to be one of the earliest affected regions showing perfusion deficits, with changes occurring before temporoparietal deficits (67). Other studies have suggested that hypoperfusion in the temporoparietal regions are more effective in distinguishing progression from MCI to AD than hippocampal perfusion (100,101). By contrast, one study using perfusion (arterial spin labelling) MRI imaging showed an increase in hippocampal perfusion in AD

individuals in comparison to controls (102). A number of different reasons could explain this discordance. The first is that the medial temporal lobe, including the hippocampus, is a small region located deep in the brain, and the low resolution inherent in perfusion SPECT brain imaging may reduce hypoperfusion detection in the region. Secondly, atrophy of the MTL in AD may contribute towards partial volume effects on perfusion SPECT imaging (103,104). Functional studies with atrophy correction however suggest that hippocampal hypometabolism exceeds volume loss in the region (100,105). Finally, the reduction in perfusion in this region could be a result of differences in average subject ages in the research samples, as supported by studies exploring perfusion pattern differences between older (>65/70 years old) and younger (<65/70 years old) AD patients (57,68,69). These studies found that younger AD subjects were more likely to have a decrease of rCBF in the posterior regions (parietotemporal and posterior cingulate), whereas older AD patients had more significantly decreased perfusion in medial temporal regions.

Hypoperfusion of the inferior parietal, precuneus and posterior cingulate regions have been shown to differentiate AD from other dementia subtypes with differing levels of accuracy. In comparison with FTD, typical AD can be distinguished by temporoparietal hypoperfusion and relative sparing of the frontal regions with good sensitivity and specificity (54,76). Other regions that are relatively spared in AD include the occipital lobe, cerebellum and basal ganglia, with frontal and anterior temporal regional perfusion usually unaffected until the later stages of the disease (62,106–108). Uncommon variants of AD can however present with patterns on imaging that differ from the typical AD pattern noted above, such as hypoperfusion and atrophy in the posterior of the brain (including the occipital lobe) in posterior cortical atrophy due to AD pathology (109,110). Frontal and temporal imaging deficits have also been shown to be present in some AD cases (111,112).

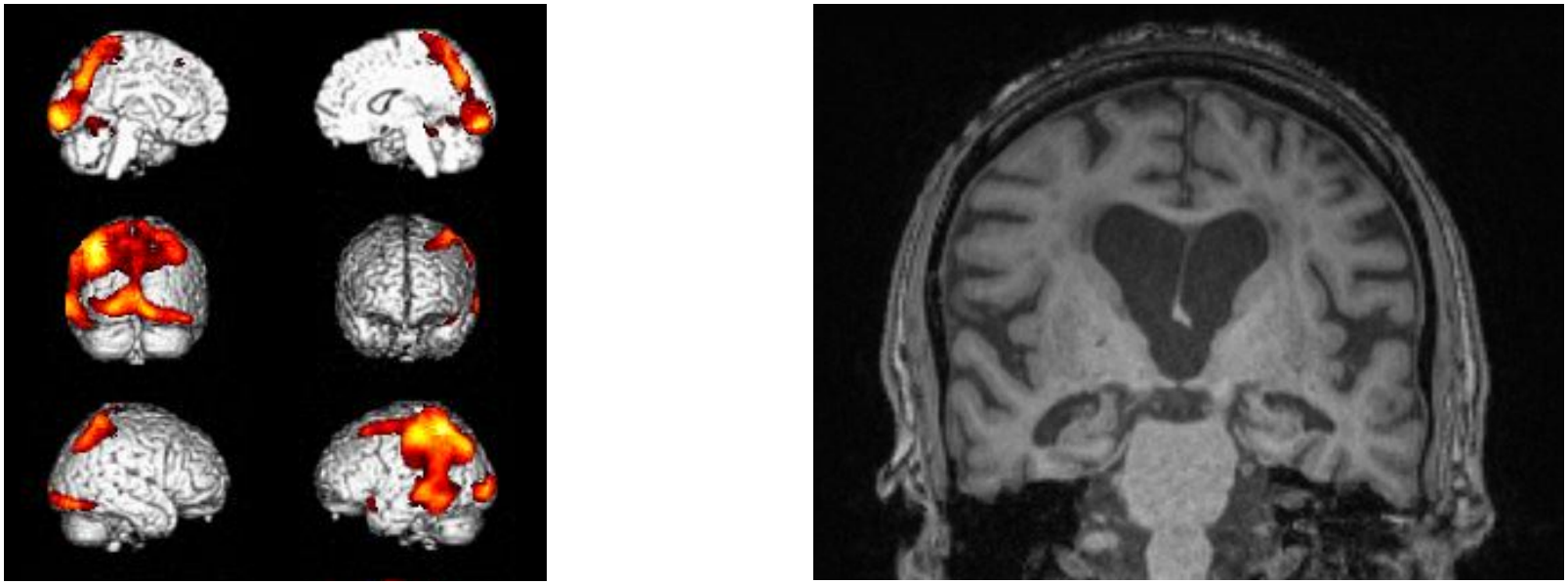


Figure 1-2 Typical Alzheimer's disease functional and structural imaging patterns

Examples of a SPECT pattern using statistical parametric mapping (left) and a structural MRI image (right) for Alzheimer's disease. Hypoperfusion of the temporoparietal and precuneus regions can be seen. Hypoperfusion is also common in the posterior cingulate. Atrophy of the medial temporal region is the most persistent structural biomarker for Alzheimer's disease, with accompanying generalised volume loss across the cerebrum (right). Please note that images shown are from separate patients.

1.3.2 Dementia with Lewy Bodies

Dementia with Lewy bodies (DLB) is the second most prevalent neurodegenerative dementia behind AD, with an estimated 10% of dementia sufferers having DLB (113). Patients with DLB present with varying levels of movement and cognitive dysfunction, with cognitive deficits (memory, visuospatial skills and attentional difficulties) and movement difficulty (rigidity, gait disturbance, tremor and bradykinesia) progressively worsening throughout the disease (28). DLB individuals most commonly present initially in distinct groups: those with cognitive or behavioural difficulty but little or no movement disturbance (similar to Alzheimer's disease) and those with movement disturbance but little or no cognitive difficulty (similar to Parkinson's disease). DLB patients show cortical Lewy body pathology (114), however also frequently show Alzheimer's disease pathology of amyloid plaques, tau tangles and vascular pathology (113). As DLB often presents with comparable clinical and pathological features to AD, Parkinson's disease (PD) and VaD, diagnosis is difficult and patients can be misdiagnosed in clinical settings (115). Early and accurate diagnosis is particularly important for providing optimum clinical management to DLB patients, with patients hypersensitive to antipsychotic medications (113).

Criteria for the diagnosis of DLB was first defined by the DLB international consortium in 1996, with an update completed in 2005 (28,116). Revised criteria include the use of brain imaging techniques including functional imaging to detect dopaminergic transporter loss in the basal ganglia and perfusion imaging to aid diagnosis. Patients with DLB show non-specific generalised atrophy across the brain, with relative preservation of the medial temporal lobe structures in comparison to Alzheimer's disease (117). Atrophy and deficits in neuronal function can however vary widely between patients.

1.3.2.1 Dementia with Lewy bodies SPECT patterns in diagnosis

1.3.2.1.1 123I-FP-CIT SPECT (DaTSCAN) imaging

The use of [123I]-2b-carbomethoxy-3b-(4-iodophenyl)-N-(3-fluoropropyl)nortropane (123I-FP-CIT) SPECT (DaTSCAN) imaging has been shown to be a highly accurate test for distinguishing DLB from AD, with an autopsy validated sensitivity of 80%, specificity of 92% and overall accuracy of

86% (118,119). DaTSCAN imaging allows qualitative visualisation and quantitative measurement of the striatal dopamine transporter availability by imaging the uptake of a radioactive tracer that binds to dopamine transporters. The striatal dopamine transporter levels are used as an indirect measurement of the nigrostriatal dopaminergic neuronal function in a patient. Tracer uptake can then be compared to a normal database to distinguish patients with reduced striatal binding of the tracer and therefore reduced striatal function. This reduction in uptake is not seen in AD patients. Although DaTSCAN imaging is the gold standard test for antemortem DLB diagnosis, it is often completed secondary to functional imaging techniques such as perfusion SPECT imaging.

1.3.2.1.2 Perfusion SPECT imaging

Individuals with DLB show a pattern of perfusion deficits similar to that seen in Alzheimer's disease, with temporoparietal hypoperfusion common (28) (Figure 1-3). This similarity in perfusion patterns reduces perfusion SPECT usefulness in distinguishing DLB from AD when using only bilateral posterior rCBF abnormality (54). There is evidence however that occipital lobe and posterior cingulate function can be used as a marker to distinguish DLB from AD. The presence of occipital lobe hypoperfusion in DLB has been suggested to be useful in classifying DLB from AD and controls (72,84,120–123), although the reliability of this sign is debated (124). More recently, it has been identified that DLB patients have relatively preserved function of the posterior cingulate in comparison to AD, in which significant functional deficits are present (125–127). Preservation of the posterior cingulate region in DLB has been combined with precuneus or occipital regions (the 'posterior cingulate island sign') to classify DLB from AD with high sensitivity and reasonable specificity in PET and SPECT studies (125,126). The combination of reduced occipital lobe perfusion, particularly of the medial regions, with preserved perfusion in the posterior cingulate can distinguish DLB from AD with good accuracy (126). Meta-analysis of perfusion SPECT papers found sensitivities of 65% to 85% and specificities of 72% to 87% for DLB detection (128).

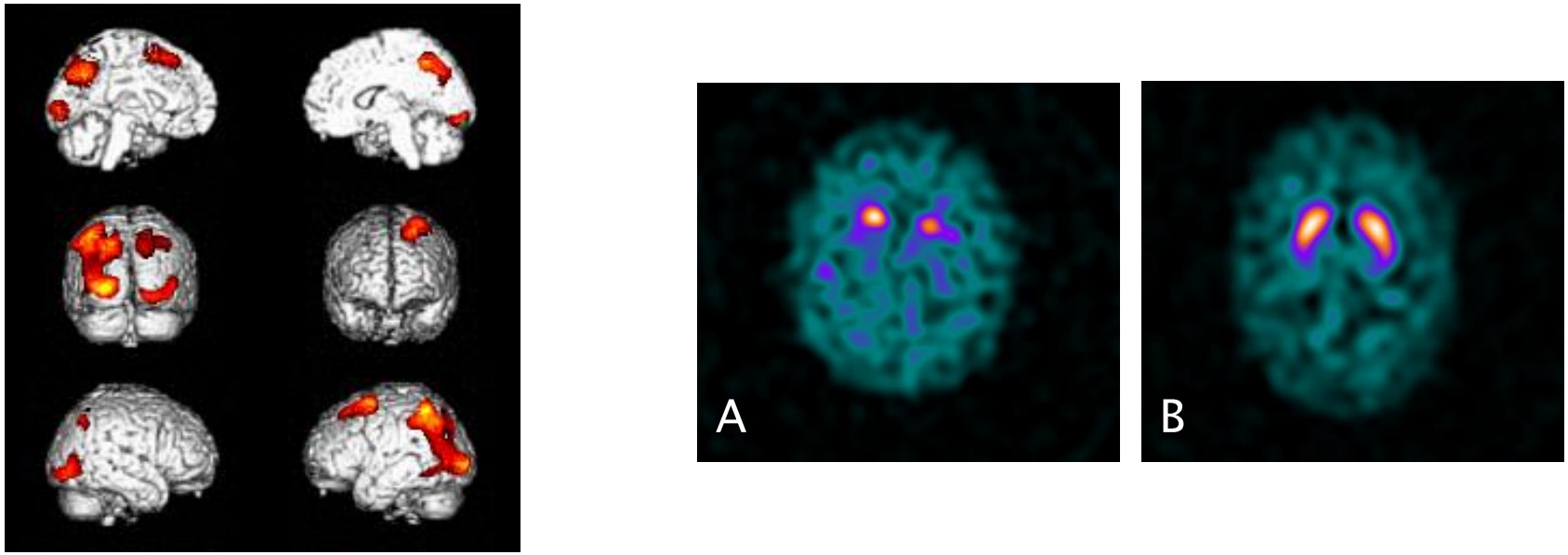


Figure 1-3 Typical dementia with Lewy bodies perfusion and dopamine transporter imaging patterns

Examples of a hypoperfusion SPECT pattern (left) and DaTSCAN (right) for dementia with Lewy bodies. Hypoperfusion of the temporoparietal and occipital regions can be seen, with relative preservation of the posterior cingulate in comparison to Alzheimer's disease. Dopamine transporter SPECT axial images using 123-I tracer are shown on the right, with reduced tracer uptake in the dopaminergic transporter rich striatum in DLB (A) compared to a normal patient (B). Please note that images shown are from separate patients.

1.3.3 Frontotemporal dementia

Frontotemporal dementia (FTD) is the third most common type of neurodegenerative dementia behind AD and DLB, and it is the second most common cause of younger onset dementia behind AD (1,2). FTD is characterised by atrophy in the frontal and temporal regions of the brain and includes both behavioural variant FTD (bvFTD) and primary progressive aphasia subtypes (PPA). Structural neuroimaging is well validated as a biomarker for FTD (19–25) and although perfusion deficits specific to FTD subtypes have been outlined, there is limited evidence to support their use as a biomarker (129). Pathology of FTD is characterised by frontotemporal lobar degeneration (FTLD) with the specific location of atrophy in the frontal and anterior temporal regions of the brain varying depending on the FTD subtype.

Behavioural variant FTD is the most common FTD subtype and is characterised by a progressive worsening of behavioural and personality changes. Frontal and/or anterior temporal atrophy are supportive of bvFTD diagnosis. Frontotemporal dementia clinical diagnostic criteria was first defined by Neary and colleagues in 1998, and an update to bvFTD criteria by international consensus in light of more recent research was completed in 2011 (130,131).

Primary progressive aphasia (PPA) is a language predominant dementia associated with atrophy and functional deficits of the frontal and temporal regions of the brain, particularly in the left hemisphere. There are three main types of PPA; semantic dementia (SD), progressive non-fluent aphasia (PNFA), and logopenic aphasia (132). Although logopenic aphasia is classified as a primary progressive aphasia, it shows Alzheimer's pathology rather than FTD pathology (133). PPA is classified with language dysfunction being the most prominent and quickly deteriorating clinical feature of the disease, which leads to impairment in activities of daily living.

The PNFA subtype is defined primarily by a loss of fluency, with effortful speech, word finding difficulties (anomia) and disturbances of syntax. On structural imaging of PNFA atrophy of the inferior frontal gyrus, left hemisphere predominant, is common (132). SD is characterised by deficits in language comprehension (semantics), naming and object knowledge, with preserved fluency and language structure (syntax). Predominant atrophy of the anterior temporal lobe is often seen in SD (132). Logopenic aphasia is

characterised by impaired single-word retrieval and repetition of phrases, speech errors and spared semantics, syntax and motor speech. Individuals with logopenic aphasia commonly show predominantly left posterior perisylvian or parietal atrophy (133).

1.3.3.1 Frontotemporal dementia perfusion SPECT pattern in diagnosis

Perfusion deficits in behavioural variant frontotemporal dementia are distinct, with medial frontal, anterior temporal and insular cortex hypoperfusion reported (55,134–136). The use of an anterior to posterior perfusion ratio has been shown to distinguish bvFTD patients from those with AD with good sensitivity and specificity (54,135). When regional hypoperfusion was compared to histologically confirmed FTD and AD, a reduction of frontal rCBF gave good diagnostic value for classifying FTD from AD (see Figure 1-4) (56). Other studies have shown good classification accuracy for FTD over AD and other dementias using frontal and temporal hypoperfusion with preservation of posterior perfusion (55,56,134,135,137). Behavioural variant FTD patients can sometimes however show temporoparietal perfusion deficits, reducing classification accuracy between bvFTD and AD (53). Studies have shown a posterior spreading of functional changes as the disease progresses (136). Posterior hypoperfusion may be explained by a proportion of FTD patients showing mixed pathology, with Alzheimer's disease type CSF protein pathology found to be present in some FTD individuals (138,139). PPA patients with AD type CSF biomarkers have been shown to have a perfusion pattern that is both posterior and frontal, compared to non-AD pathology PPA patients who show temporal pole pathology (139,140). This highlights the heterogeneity found in each dementia subtype and the difficulty it poses for differentiating dementia subtypes from one another.

The use of perfusion imaging to classify PPA is not well validated, however a review exists that covers the topic (21). Non-fluent progressive aphasia has been shown to have left frontal or fronto-temporal hypoperfusion (141), with left predominant posterior fronto-insular hypoperfusion supporting PNFA criteria for diagnosis (132). Semantic aphasia has been seldom explored by perfusion SPECT, however FDG PET studies show predominantly left sided hypometabolism with posterior cingulate preservation of function (21,142,143). Criteria for diagnosis identify predominant anterior temporal hypoperfusion on SPECT as supportive of a semantic aphasia diagnosis (132).

The logopenic variant of PPA has shown inferior parietal with left posterior temporal hypoperfusion in comparison to healthy controls (132,133).

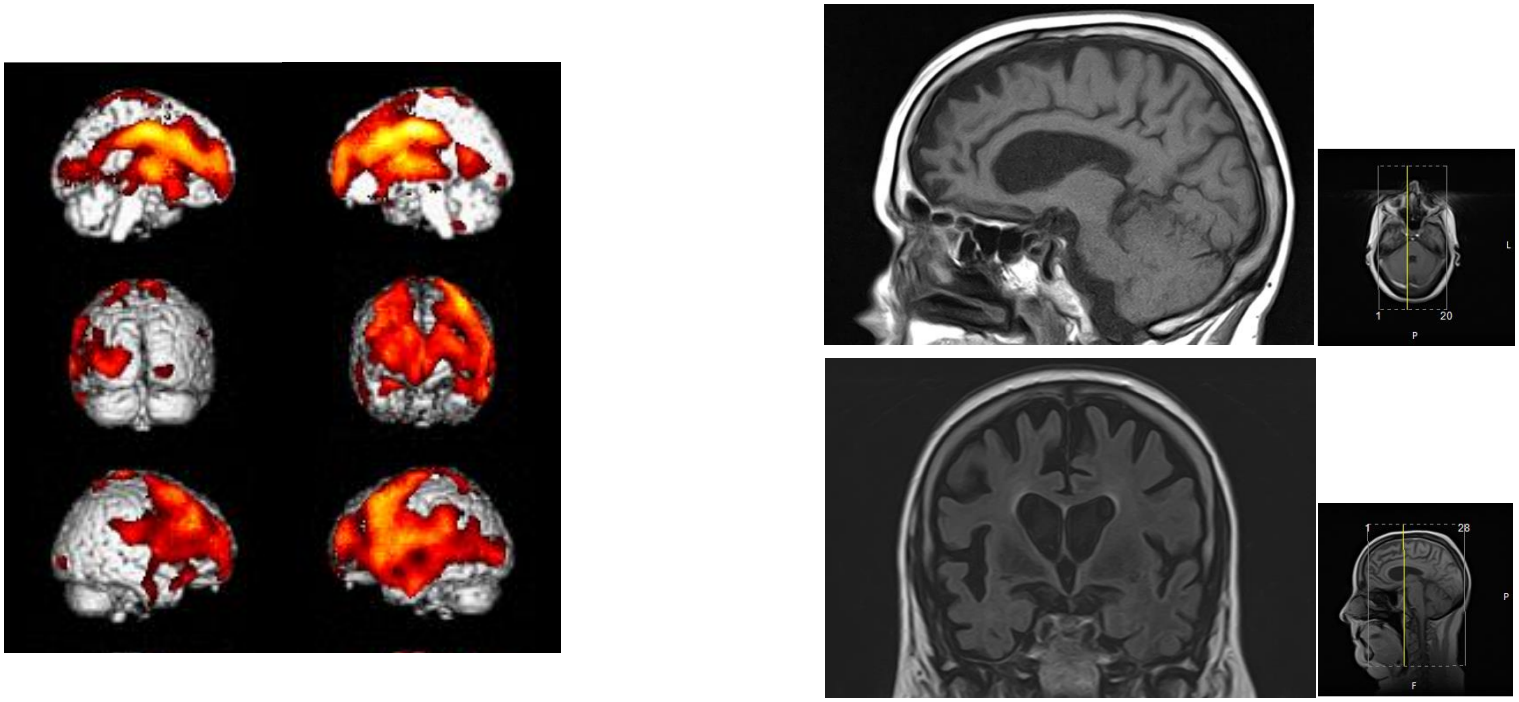


Figure 1-4 Typical behavioural variant frontotemporal dementia perfusion and structural patterns

Examples of a hypoperfusion SPECT pattern using statistical parametric mapping (left) and a structural MRI image (right) for behavioural variant frontotemporal dementia. The frontal regions are preferentially affected (red areas indicate hypoperfusion) with posterior perfusion relatively preserved in comparison to normal controls on SPECT. Atrophy of the frontal and temporal lobes also occurs in bvFTD (right; sagittal and coronal views). Please note that images shown are from separate patients.

1.3.4 Vascular Dementia

Vascular dementia (VaD) or vascular cognitive impairment (VCI) is a slowly progressive or non-progressive disease where patients show varying levels of cognitive dysfunction due to cerebrovascular brain injury, such as infarcts, microinfarcts or microbleeds that result in a reduced blood flow to specific areas of the brain (144–146). This reduced blood flow affects brain structure and cognition. There are multiple subtypes of vascular dementia, although exact aetiology and pathophysiology behind the disease is still debated (146). Vascular dementia is often seen in conjunction with other dementia pathologies, particularly Alzheimer's disease (144,147,148), and around a third of dementia cases show significant vascular pathology at autopsy (149). Cognitive impairment seen in vascular dementia is variable, more so than other dementias such as Alzheimer's disease and frontotemporal dementia, and depends on the cortical or subcortical regions affected by pathology. This can often make it difficult to diagnose using cognitive assessment alone, however multiple diagnostic guidelines exist to aid clinicians in VaD diagnosis (150–153). Structural imaging to detect white matter hyperintensities (WMHs) and infarcts in the brain is an *in vivo* technique used to identify vascular changes (144).

1.3.4.1 Vascular dementia perfusion SPECT pattern in diagnosis

Perfusion deficits in individuals with VaD are not clearly defined and SPECT imaging is not included in current guidelines for VaD diagnosis (154). Perfusion SPECT has however been shown to be useful in differentiating VaD from AD (54,55,71,75,76) and other dementias (54,55,155), with a patchy or bilateral anterior pattern of perfusion loss seen in VaD compared to AD (156,157) (Figure 1-5). This bilateral frontal pattern can provide a moderate increase in likelihood of VaD over AD, with a bilateral posterior pattern increasing odds of AD over VaD (54). Meta-analysis studies have shown pooled sensitivities of 71% to 74% and specificities of 72% to 76% for distinguishing AD from VaD with HMPAO SPECT (75,76). Perfusion SPECT was however of little use in differentiating VaD from frontotemporal dementia or progressive aphasia, with only small likelihood ratios seen (54). A number of papers have also failed to find a consistent hypoperfusion pattern for VaD (55,158,159).

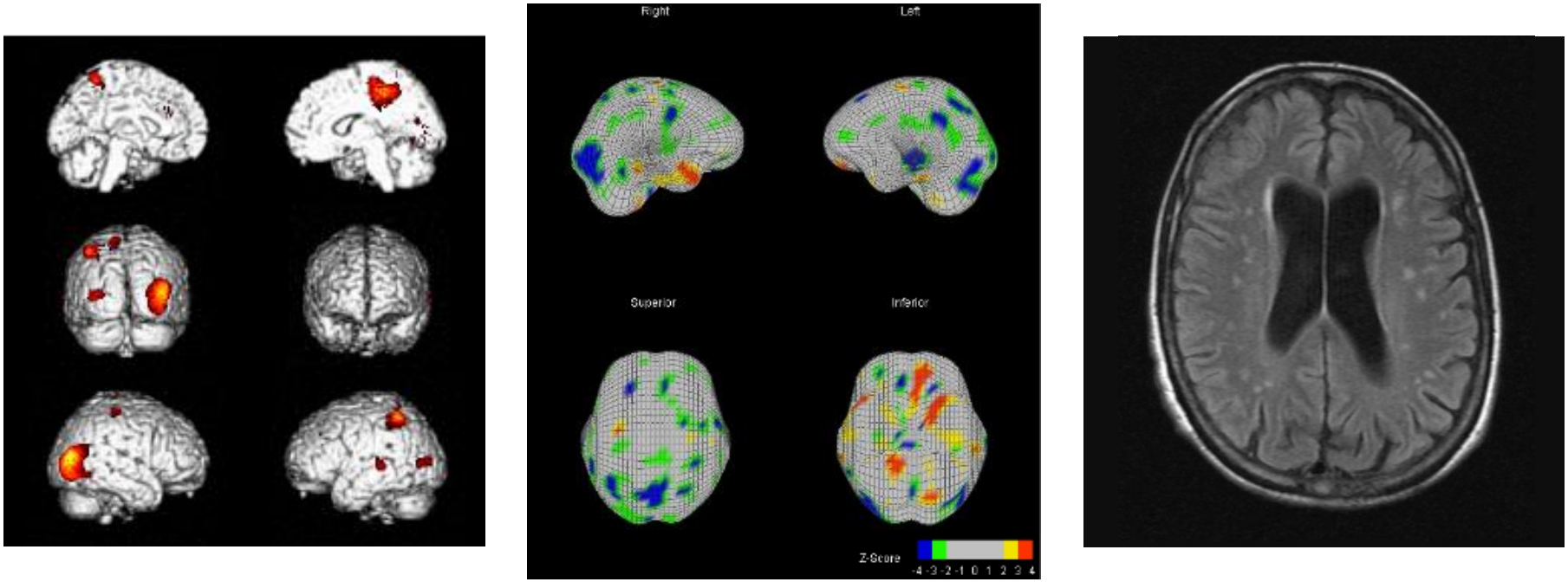


Figure 1-5 Typical vascular dementia functional and structural imaging patterns

Examples of a hypoperfusion SPECT pattern using statistical parametric mapping (left), a perfusion SPECT using EXINI 3D-SSP analysis (middle), and FLAIR MRI imaging (right) for vascular dementia. For rCBF, patchy hypoperfusion is seen across the cerebrum. Structural imaging using FLAIR MRI imaging shows white matter hyperintensities indicating moderate small vessel ischaemia (right). Please note images shown are from separate patients.

1.4 Perfusion SPECT imaging patterns in dementia prognosis

The usefulness of perfusion SPECT in prognosis is less clear than its diagnostic ability, with longitudinal studies showing varying levels of effectiveness for SPECT patterns in predicting decline. Nearly all of these studies look at the ability of perfusion SPECT to predict progression to AD from MCI, with both retrospective and prospective samples used. Other studies comparing longitudinal decline of regional perfusion in disease progression have also been completed (82,85–87,160–164).

1.4.1 Prognosis and prediction of decline in Alzheimer's disease

There are a number of longitudinal studies that compare baseline perfusion SPECT imaging of MCI patients who convert to AD (progressive or pMCI) from those with cognitive impairment who do not cognitively worsen (stable or sMCI) (73,78,79,85,92,96,99–101,161,165–171). Reduced rCBF in the temporoparietal region including angular and supramarginal gyri (73,79,99–101,161,165,166,168,171,172), posterior cingulate (96,167,171), medial temporal lobe including hippocampus and parahippocampus (96,99,169,171,172), superior temporal lobe (165,168), frontal lobe (79,101,168) and precuneus (79,100,169) regions has been found in pMCI when compared to patients with sMCI. Increased perfusion in pMCI compared to sMCI in frontal regions has also been suggested (73,168). Three studies however found no difference in perfusion values between progressive and non-progressive MCI (85,92,170).

Multiple studies have completed logistic regression, discriminant or receiver operating characteristic (ROC) curve analysis to observe regions that best predict conversion to AD. The posterior cingulate alone was found to predict conversion to AD with reasonable accuracy (ROC area under the curve 0.74 to 0.87) (90,167,168,171). The left parietal, inferior parietal, angular gyrus and precunei have each been found to have good sensitivity (63% to 90%) and overall accuracy (68% to 75%) in predicting conversion (73,100). The temporoparietal and anterior temporal regions were also shown to have high prediction accuracy (63). Different combinations of the hippocampus, posterior cingulate, anterior cingulate, anterior thalamus, parietal, precuneus, frontal

and temporal regions have shown accuracy for predicting MCI conversion to AD ranging from 59% to 89% (78,79,96,99,101,161,166). A meta-analysis of longitudinal perfusion SPECT studies showed a pooled sensitivity of 84% and specificity of 70% for predicting MCI conversion to AD (173).

Perfusion SPECT has also been shown to be useful in both predicting time to conversion of AD from MCI and AD survival time. Survival analysis completed by Hansson et al (2009) using an inhaled ^{133}Xe tracer for perfusion SPECT imaging showed progression to AD from MCI was more likely in those with significantly decreased perfusion in the parietal cortex, with a hazard ratio of 3.1 (165). Those who had parietal perfusion loss and abnormal CSF AD biomarkers declined the fastest (hazard ratio 24.3). Survival analysis by Devanand et al (2009) also found when grouping patients using their rCBF values that low rCBF in the parietal cortex and medial temporal lobe significantly increased the hazard of conversion to AD (99). Survival analysis in AD patients by Jagust et al (1998) using ^{123}IMP tracer and manually drawn ROIs found that right parietal lobe rCBF predicted survival in a proportional hazard model, with an average time to survival of 53 months for those in the lowest right parietal rCBF tertile, compared to 89 months in the highest right parietal tertile (174). Significant correlations have also been shown between parietal (165,175) and temporal (176) rCBF with neuropsychological decline as measured by the mini-mental state examination in both AD and MCI patients. MMSE decline and rCBF have however not consistently shown relation (174).

There are several limitations to these longitudinal studies. The first is sample size, with only one paper having a sample with more than 50 subjects in the smallest group (165). Results from these small sample studies may not be generalizable. In addition, many of the studies use a circular argument, selecting variables for their discriminant or logistic analyses from t-tests or non-parametric group comparisons that show the most significant differences between groups. Selecting regions from the same sample that they are tested on may artificially inflate the accuracy of the test for predicting conversion. Only one of the longitudinal studies used a small validation sample (63).

1.4.2 Prognosis and prediction of decline in non-Alzheimer's dementia

The longitudinal papers noted above were completed on either AD patients or MCI patients that either progressed to AD or remained stable. Other dementia

groups were excluded from the analyses. This review identified two longitudinal prospective papers that investigated the usefulness of SPECT imaging for the differential diagnosis of clinically ambiguous dementia (54,177). Boutoleau-Bretonnière et al (2012) found that baseline perfusion SPECT imaging gave 78% sensitivity and 62% specificity for subsequent AD diagnosis at 2 years using temporoparietal deficits. SPECT prediction of frontotemporal dementia using frontal hypoperfusion pattern had a sensitivity of 73% and specificity of 78%. A normal SPECT perfusion pattern had very high specificity (91%) for a psychological diagnosis but low sensitivity (43%). A study by Talbot et al (1998) prospectively followed a large clinical sample (N=363) for 1 to 6 years to obtain dementia patterns for AD, DLB, VaD, FTD and progressive aphasia (54). Talbot et al produced likelihood ratios of individual patterns of HMPAO rCBF as assessed by visual analysis for pairwise disease group comparisons. However, neither Boutoleau-Bretonnière et al (2012) nor Talbot et al (1998) explored the usefulness of perfusion SPECT to predict future decline.

We identified only a single study that explored the utility of SPECT imaging to predict decline in a non-Alzheimer's disease or mixed clinical sample (73). Huang et al (2003) followed 82 MCI individuals for an average of two years and used discriminant analysis to test the ability of perfusion SPECT to predict decline to any dementia. Huang et al (2003) did not however explore decline rates or survival for perfusion SPECT patterns in their sample. A study that investigates the relationship between different patterns found in a mixed clinical sample and rates of decline could help improve detection of individuals who are at highest risk of decline. This knowledge could aid clinicians in directing care to those at highest risk of poor outcomes.

1.5 Limitations of the evidence for use of perfusion SPECT in dementia

1.5.1 Perfusion SPECT research heterogeneity

Reviews and meta-analysis studies highlight the lack of high-quality modern studies available on perfusion SPECT utility in dementia diagnosis, with research using outdated scanners and reconstruction methods combined with analysis techniques that are not useful for the clinician. Many studies also

make conclusions on perfusion SPECT usefulness that are limited by small sample sizes that are not generalizable to clinical situations. The few modern studies available in the literature often fall just below the requisite 80% accuracy level recommended for clinical biomarkers, however often still show that the technology is clinically useful in diagnosis. There is also a large range of conclusions on the usefulness of perfusion SPECT in dementia, with individual studies showing a range of sensitivities and specificities from 40% to 100% depending on methods used. There are a vast number of sources of potential heterogeneity in perfusion SPECT research.

1.5.1.1 Sample

Sample collection often varies widely, with retrospective and prospective data collection, and cross-sectional and longitudinal analysis completed. In addition, due to multiple clinical criteria for each dementia subtype, criteria used to classify dementia can differ. Where meta-analyses cover a large span of dates, old clinical criteria for dementia subtypes are often not as accurate in classification as current criteria, with improvements in specificity and identification of disease phenotypes. The disease sample investigated often varies and can cover either a single subtype or a mixed sample including multiple subtypes. The source of subjects can also have an effect on the rate of conversion to dementia in mild cognitive impairment cases (178). There are only a few studies that use gold standard histopathological confirmation of dementia disease, with the majority using clinical follow up or cross-validation against another biomarker. Sample age is another source of heterogeneity, with rCBF directly correlating with age. Research has also shown that perfusion SPECT shows different effectiveness depending on whether the sample is younger (under 65 years of age) or older (over 65 years of age), with different regions of interest being affected in each group (57,68,69,179). Additionally, samples must be controlled for the proportion of men and women in both the disease and control samples, as women have increased rCBF levels in comparison to men (96,180). Ideally, genetic risk factors such as apolipoprotein E (APOE) would also be controlled for in the sample, with differences in perfusion between carriers and non-carriers (82,87). This information is however not always ready available in clinical samples. Finally, different studies use individuals with different times from symptom

presentation to their scan date, which could lead to further variability. When subjects are followed longitudinally, outcome measures also differ.

1.5.1.2 Image collection and processing

Many sources of variability arise from image collection and processing. This includes physical aspects of the image collection such as the tracer used and amount of tracer, the type of camera used and whether it is a single, dual or multiple head camera and the type of collimator used. Other aspects, such as the number of projections, the acquisition time and patient preparation (time of injection before scanning, whether the patient was scanned with eyes closed or open) can also differ. The image reconstruction (whether filtered back projection or ordered subset expectation maximisation method), correction methods (attenuation and/or scatter), filters and matrix size can also vary.

Once the image is reconstructed, pre-processing methods such as those carried out in SPM and 3-D SSP (as described above) have multiple options including normalisation template, smoothing FWHM size and region used for count normalisation. Once pre-processed, analysis methods vary, including visual analysis, semi-quantitative (automated or manual regions of interest, choice of thresholds used) and quantitative methods. Some studies also implement partial volume correction for atrophy, whereas other studies do not.

1.5.1.3 Meta-analysis

There is an inherent risk in meta-analysis on perfusion SPECT imaging in dementia that the need to restrict the input of studies to control for heterogeneity can result in an analysis which only considers a small subsection of the literature. Studies using mixed clinical samples are often excluded from analysis, which can lead to the exclusion of genuinely useful translational studies from the meta-analysis. On the other hand, inclusion of studies with large heterogeneity of methods can result in 'watered down' results of quality research. A meta-analysis of SPECT studies predicting decline from MCI to AD has highlighted these issues and found strong evidence of between-study heterogeneity in SPECT research (22). This heterogeneity contributes towards the discussion of the usefulness of perfusion SPECT imaging in dementia, particularly in relation to other modalities such as metabolic PET imaging (FDG PET).

1.5.2 Perfusion SPECT or metabolic PET?

Perfusion SPECT or metabolic PET imaging are both recommended for use in multiple dementia diagnosis guidelines (listed above) and due to the close biological link between perfusion and metabolism in the brain, patterns on both modalities are often similar if not identical. The heterogeneity and age of perfusion SPECT research however makes it difficult to fully assess the usefulness and reliability of the technology in clinical diagnosis, and therefore some diagnosis guidelines recommend the use of PET imaging over SPECT imaging where possible (47,49).

Perfusion SPECT and metabolic PET both have advantages and disadvantages over one another. SPECT is more widely available, cheaper and has a more stable tracer than FDG PET imaging (181). Due to the short half-life of the FDG PET tracer (110 minutes), a nearby cyclotron is required to produce the tracer close to the time of use. ^{99m}Tc tracers such as HMPAO however can be produced on site using a generator through a process of decay of a radioactive tracer with a half-life of 66 hours (^{99}Mo). This allows time for transportation of the tracer before it is required. FDG PET imaging has improved spatial resolution over perfusion SPECT (4-6mm compared to 8-16mm) and it is thought that FDG has a closer affinity to metabolism than SPECT technetium tracers have to cerebral blood flow (182). FDG PET is often quoted as having increased diagnostic accuracy over perfusion SPECT, particularly using purely visual analysis (35,183-185), however the extent of this is debated (186). Despite increased accuracy of FDG PET over perfusion SPECT, FDG PET results can be influenced by blood glucose levels (187) and provides no information on the cerebral vascular health of the patient.

Numerous papers have investigated the diagnostic accuracy of perfusion SPECT and metabolic PET individually in classification of dementia subtypes and multiple review papers are available (22,75,128,158,184). One particularly interesting review paper by Frisoni et al (2013) completed meta-analysis on imaging biomarkers available for dementia diagnosis, grouping results by technology used and analysis technique applied (22). They found that although metabolic PET imaging did provide increased diagnostic usefulness through improved likelihood ratios over perfusion SPECT, metabolic PET imaging showed the greatest variability in likelihood ratios both between and within analysis methods. This variability within PET imaging was greater than the

variance in likelihood ratios between perfusion SPECT and metabolic PET, suggesting that without standardisation of analysis techniques, PET imaging may not provide improved diagnostic accuracy over perfusion SPECT. Other meta-analysis using semi-quantitative and quantitative analysis have shown FDG PET to be moderately better at predicting conversion to AD from MCI than perfusion SPECT, with around 89% and 84% sensitivity and 85% and 70% specificity for FDG PET and HMPAO SPECT respectively (173). In discrimination of AD from other dementias, meta-analysis has shown the area under the ROC curve (AUC) for perfusion SPECT and FDG PET to also be similar, with PET showing slight superiority (0.86 for SPECT to 0.91 for PET) (188).

Direct head to head comparison studies of accuracy in dementia diagnosis between perfusion SPECT and metabolic PET have been completed with conflicting results (97,184–186,189). Using visual analysis for both FDG PET and perfusion SPECT (using a 99mTc-ECD tracer), Ito et al (2014) found similar patterns of uptake in frontal, temporal and occipital regions with no marked differences between the modalities diagnostic abilities, although correlation between PET and SPECT occipital tracer uptake was poor (186). Poor correlation between metabolic PET and perfusion SPECT in the occipital region could explain an increased sensitivity and specificity found for FDG PET in distinguishing AD from DLB over perfusion SPECT (97). To our knowledge, there have been no studies that directly compare the diagnostic utility of the two modalities on the same sample using quantitative methods of analysis such as statistical parametric mapping.

Although studies suggest improved accuracy of FDG PET over perfusion SPECT, most still show that perfusion SPECT is useful in diagnosis and SPECT is suggested when FDG PET is unavailable (128,190). The wide availability of perfusion SPECT in comparison to FDG PET and its ability to provide information above that which can be obtained through standard clinical assessment, neuropsychology and structural imaging, would suggest it still has a place in aiding dementia diagnosis.

1.6 Utility of perfusion SPECT in clinical practice

1.6.1 Clinically relevant samples

As previously discussed, research into biomarkers is often completed in select samples where the disease group is well-defined and characterised. These well-defined disease subjects are also commonly compared to controls without imaging deficits. Clinicians frequently face the issue of trying to distinguish one dementia subtype from another, and for a biomarker to show its true sensitivity and specificity in clinical practice, research that differentiates between different dementia subtypes and non-dementias from one another must be used. There have been few studies that have validated perfusion SPECT biomarkers in large naturalistic clinical samples that represent real-life practice.

1.6.2 Assessment of care needs

Research on perfusion SPECT imaging has been confined to its validity in prediction of diagnosis and prognosis, and its ability as a tool to assess care needs has not been explored. The ability of perfusion SPECT to predict the care needs of the patient and/or their caregiver would have utility in clinical practice, allowing timely implementation of care provisions to help both the patient and their family throughout the progression of the disease. Although research on common symptoms in each dementia subtype as they progress is available, symptoms are dependent on location and extent of pathological change in the brain. Individuals with atypical presentations or mixed type pathology may therefore present with symptoms that are not expected for the subtype they are diagnosed with. Brain imaging may have predictive value for future symptoms and expected care needs for a patient, such as the amount of burden a patient may place on their caregiver. The utility of perfusion SPECT imaging to predict these needs is currently unknown.

1.6.3 Clinician diagnostic confidence

Research investigating the utility of SPECT perfusion imaging in improving diagnostic confidence is limited, with only a single study investigating clinician diagnostic confidence in perfusion SPECT identified (57). Van Gool et al (1995)

prospectively studied 110 older individuals who had suspected dementia with both standard clinical examination and perfusion SPECT, with visual temporoparietal deficits used as a biomarker for AD. They found that although clinicians expected perfusion SPECT to aid the diagnostic process in 26% of patients, at final diagnosis clinicians rated that perfusion SPECT influenced their level of confidence in only 8% of the subjects. This study by Van Gool et al (1995) was first published over twenty years ago and therefore uses older methods of image collection and analysis (visual interpretation and manually drawn regions of interest). A study by Jagust et al (2001) investigating the change in accuracy of clinical diagnosis with the addition of visually inspected perfusion SPECT, found an increase from 84% correctly diagnosed according to pathological confirmation to 92% with positive SPECT (191).

We identified no studies exploring perfusion SPECT use with newer sensitive semi-quantitative or quantitative analysis (such as SPM) and change in clinician diagnostic confidence. The increased sensitivity and specificity that can be achieved by perfusion SPECT imaging using these methods would suggest that perfusion SPECT imaging would have a larger influence on clinician confidence than that found in the study by Van Gool et al (1995). A study that quantitatively investigated diagnostic confidence both before and after imaging would allow comparison between clinician perceived usefulness of the technology and a quantitative diagnostic confidence change. This method was used by Ossenkopple et al (2013) to investigate clinician confidence change with FDG PET imaging, with an increase in diagnostic confidence of 16%, a change in diagnosis in 23% of patients and an improvement of the clinicians understanding of their patient's disease in 64% of cases after imaging found (192). Elucidation of the utility of perfusion SPECT imaging with semi-quantitative analysis to alter the diagnostic confidence of clinicians would have implications for clinical imaging choice in diagnostically ambiguous cases of patients with memory complaints.

1.7 Conclusion

Research on neuroimaging biomarkers to aid dementia diagnosis and prognosis has soared in recent years, with the advent of amyloid-beta and

other molecular imaging techniques that allow visualisation of specific pathological pathways in dementia. Novel image analysis techniques have also been shown to give a higher accuracy in disease identification. There has however been a lack of high quality studies that focus on clinical translation of these novel technologies and analysis techniques. In addition, few studies use naturalistic samples that are representative of the heterogeneous clinical population seen in the real world. There has also been little focus on how rCBF SPECT, which is commonly available to aid dementia diagnosis, can be improved by the use of newer image analysis techniques.

Despite being a longstanding technology, perfusion SPECT imaging is still a useful technique that gives information above that which can be obtained from clinical assessment and structural imaging alone. The technology is cheaper, easier to implement and more widely available in the UK than metabolic PET, however due to the heterogeneity of perfusion SPECT imaging techniques and prior research it is very difficult to assess its accuracy in dementia diagnosis. The use of imaging techniques such as voxel based morphometry and region of interest analysis can increase the effectiveness of perfusion SPECT in dementia diagnosis. Although numerous papers exist that evaluate perfusion SPECT imaging in dementia, studies that evaluate its diagnostic and prognostic utility in clinical cognitive impairment samples using these semi-quantitative measures are sparse. Those that do rarely use analyses that can evaluate abnormalities in the individual, limiting their usefulness for clinical situations. In addition, evaluation of the utility of perfusion SPECT with these analysis techniques to predict care needs and aid a clinician's diagnostic confidence has not been completed.

Further research that validates the use of perfusion SPECT imaging patterns using quantitative and semi-quantitative analysis in clinical samples where diagnostic ambiguity exists is needed. The utility of perfusion SPECT imaging patterns to aid diagnosis, improve clinician confidence in diagnosis and predict decline in the individual in clinical settings is still to be addressed.

1.8 Aims and objectives of the thesis

The previous sections outline the current knowledge and limitations surrounding perfusion SPECT imaging use in dementia and highlight the need for updated clinical validation of the modality in dementia disease diagnosis and patient prognosis. This thesis presents a number of translational studies that use naturalistic, clinical samples to validate prior work and investigate the real-world utility of perfusion SPECT imaging using modern methods of analysis for dementia diagnosis.

The aim of this research is to provide clinical benefit by maximising the information that can be utilised from perfusion SPECT, with specific objectives of: 1) enhancing knowledge of clinical perfusion patterns that indicate dementia pathology, or likely decline; and 2) providing evidence for the utility of perfusion SPECT imaging in clinical practice.

The thesis has a particular focus on the following currently unanswered questions:

1. Is quantification of perfusion SPECT valid and reliable for disease diagnosis in a clinical environment?
2. Can additional information above diagnosis be gained from routinely collected imaging to aid patient clinical care?
3. How does perfusion SPECT imaging influence clinician confidence and diagnostic decision-making?

There are five studies presented in this thesis. The first two studies define imaging perfusion patterns that can predict independent clinical biomarkers (DaTSCAN imaging and CSF AD biomarkers). Perfusion SPECT patterns seen in the individual are defined and their association with pathology are investigated.

The third study explores the ability of perfusion SPECT imaging patterns to predict patient functional decline using a naturalistic longitudinal observational study of individuals with cognitive complaints. Survival probabilities of individuals with abnormal (both globally and regionally) and normal imaging are compared.

The fourth study investigates the relationship between perfusion SPECT imaging patterns and caregiver burden, defining patterns on imaging that can aid prediction of patients that are at high risk of placing high burden on caregivers.

The fifth and final study assesses the utility of perfusion SPECT in clinical practice. Diagnosis and clinician confidence in their diagnosis was compared both before and after perfusion SPECT imaging to assess utility in influencing clinical decisions in the diagnostic process.

Chapter 2: Methods

Research presented in this PhD uses samples from two independent studies and one service improvement study of patients with cognitive complaints. These studies have been designed to utilise data obtained in the process of clinical care, allowing a naturalistic sample to be collected that represents real life clinical situations. The independent studies were approved by the relevant NIHR ethics committees and University Hospital Southampton research and development department (as detailed below) and the service improvement study approved by University Hospital Southampton Nuclear Medicine department. This chapter presents an overview of the methods of data collection and analysis, with further detail for each study provided in subsequent chapters.

2.1 Retrospective Brain Imaging in Dementia (RetroBraIID) study

The RetroBraIID study allowed retrospective clinical data from patients with cognitive complaints who had been seen in University Hospital Southampton NHS Foundation Trust (UHS) to be obtained and used for research purposes. Participants in the study fell into two non-exclusive groups:

1. Patients referred for nuclear medicine brain imaging for cognitive complaints (HMPAO or DaTSCAN single photon emission computed tomography) or cerebrospinal fluid neurodegenerative biomarkers completed at UHS
2. Patients who were seen in the UHS Cognitive Disorders Clinic for cognitive assessments, behavioural and functional tests

Patients included in the study were between the ages of 18 and 100 years old and seen with a suspicion of dementia. Those who were seen at the UHS Cognitive Disorders Clinic underwent standard dementia diagnostic work-up including a detailed history, physical and neurological examinations and screening cognitive assessments. Referrals for HMPAO SPECT imaging came from the UHS Cognitive Disorders Clinic and other dementia specialists in the

Wessex region. As the study was a retrospective review of existing data, no contact was made with any patients and all data and images used was pseudonymised to researchers outside the patient's normal clinical team.

Clinical data including biomarker and imaging results were obtained from the UHS nuclear medicine department clinical database, clinical picture archiving and communication system (PACS) and/or cognitive service clinical records.

The RetroBraIID study was approved by NRES Committee South Central Hampshire A/B (reference 14/NE/1252) and UHS Research and Development department (reference number NEU0254). The RetroBraIID study protocol (Version 1.5, Date 28 October 2014) can be found in the Appendices.

2.2 The Brain Imaging in Dementia (BraIID) study

The Brain Imaging in Dementia study is an ongoing observational prospective follow up study of patients referred for diagnostic HMPAO SPECT imaging. The study aims to maximise the use of imaging data acquired in the course of providing routine clinical care and combine this with long-term follow up information on individuals with cognitive complaints in order to improve diagnostic and prognostic accuracy for the individual.

All patients who are scheduled to have or have had HMPAO SPECT imaging completed at UHS as part of their clinical care due to cognitive complaints are offered the opportunity to take part in the study. Patients included in the study are between the ages of 18 and 100 years old and identified through the nuclear medicine department referrals list. At consent, patients are asked to complete a demographics questionnaire (see Appendices). If patients do not have the capacity to consent to take part in the study, they can be recruited with the use of a personal consultee.

Carers of patients enrolled are also asked to join the study and provide longitudinal follow up information on the patient. This includes a clinical staging by telephone Clinical Dementia Rating scale (CDR) completed by a trained researcher and a companion completed CDR (193), behavioural assessment using the Cambridge Behavioural Inventory (CBI) (194,195), functional activity assessment using the Functional Activities Questionnaire

(FAQ) (196) and caregiver burden as assessed by the Zarit Burden Questionnaire (Zarit) (197). Detail on each of these questionnaires is provided below. These questionnaires are completed at the time of consent (baseline) and at six monthly time points until the end of the study. The use of caregiver follow-up reduces burden on the patient participant throughout the study.

The BrainID study was approved by NRES South Central Hampshire A Research Ethics Committee (reference 15/SC/0231) and UHS Research and Development department (reference number NEU0262). The BrainID protocol (Version 1.8, 21 December 2015), invitation to participate letter (Version 1.3, 21 December 2015), Patient Information Sheets for patient (Version 1.5, 01 February 2017), companion (Version 1.5, 01 February 2017) and consultee (Version 1.5, 01 February 2017), patient and companion consent (Version 1.4, 01 February 2017) and consultee forms (Version 1.4, 01 February 2017) and GP letter (Version 1.0, 19 September 2014) can be found in the Appendices.

2.3 Clinician attitudes towards dementia investigations (CADI) service improvement study

The CADI study was a service improvement study completed to assess the perceived usefulness of HMPAO SPECT imaging by referring clinicians. The study assessed referring clinician confidence in the diagnosis and current diagnosis both before and after HMPAO SPECT brain imaging through a two-part postal questionnaire. Additionally, clinicians were asked after imaging to rate whether they felt the imaging contributed towards their diagnostic process and improved, confused or did not contribute towards their understanding of the patient's disease. Further information on the questionnaires used for the CADI study and the analysis completed is detailed in Chapter 7. Examples of the questionnaires sent to clinicians both before and after imaging can be found in the Appendices.

The CADI study was approved by the Nuclear Medicine department and Medical Physics and Bioengineering department at UHS and underwent peer review within the Ageing and Dementia theme of the NIHR Collaboration for Leadership in Applied Health Research and Care (CLAHRC) Wessex. As the study was completed internally to the cognitive service and nuclear medicine

department in UHS and as a service improvement study, no NRES approval was required or sought.

2.4 Data collection

2.4.1 Dementia rating scales

Dementia rating questionnaires were collected throughout the BrainID study to provide information on patient dementia severity, behavioural and psychiatric symptoms, function (activities of daily living) and caregiver burden. These questionnaires have been validated clinically and are currently used to aid diagnosis and care in the cognitive clinic at UHS.

The Clinical Dementia Rating (CDR) scale is a staging measure that rates the patient's cognitive performance in six domains: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. Each domain is rated on a five point scale of impairment as follows: 0, no impairment; 0.5, questionable impairment; 1, mild impairment; 2, moderate impairment; 3, severe impairment. A global or sum of boxes (SOB) score is then calculated to provide an overall measure of severity, with both scores providing accurate dementia staging in AD samples (198). The global score is calculated using an algorithm, and the SOB score is calculated by summing the domain scores (199).

The Cambridge Behavioural Inventory (CBI) is an 81 item questionnaire that investigates frequencies of problems in memory, orientation and attention, everyday skills, self-care, mood, beliefs, challenging behaviour, disinhibition, eating habits, sleep, stereotypic behaviours, motivation and insight. It has been shown to provide discrimination between a range of neurodegenerative disorders, has good test-retest reliability and shows good validity against other commonly used instruments such as the Neuropsychiatric Inventory (NPI) (194,200). The wide range of issues covered in the questionnaire and the distinct profiles shown between dementia subtypes aids classification of cognitively impaired individuals and highlights cognitive and behavioural deficits that may not be detected in clinic visits.

The Functional Activities Questionnaire (FAQ) is a 10 item tool used to assess independence in activities of daily living in cognitive impairment and dementia. Caregivers are asked to rate the patients independence on a scale of 0 (independent) to 3 (dependent) in tasks necessary for independent living, such as shopping alone or preparing a balanced meal. The questionnaire has been shown to have high reliability and is sensitive to change in cognitively impaired individuals (201,202).

The Zarit Burden Interview (ZBI) is considered a principal instrument in measuring caregiver burden in dementia (203). The instrument includes items addressing the effects of caregiving on multidimensional aspects of the caregiver's life including areas of quality of life, health and in eliciting negative thoughts/emotions. The revised 22 item version of the ZBI, which has been shown to possess sound psychometric properties of reliability and validity, was used in the BraIID study (204).

2.4.2 Image collection and processing

2.4.2.1 Perfusion SPECT imaging

Perfusion SPECT imaging was completed in all cases using a ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO) tracer. Patients were imaged while awake with eyes open in a darkened room. Imaging of patients was completed on one of two gamma cameras:

1. GE (SMV) DST-XL dual-head gamma camera equipped with low-energy, high resolution collimators
2. GE INFINIA dual-head 3/8 HK4 gamma camera with CT correction, equipped with low-energy, ultra-high resolution collimators

Acquisition differed slightly for each camera. For the GE (SMV) DST-XL camera an elliptical orbit as close as possible to the head was used and 128 projections were acquired, each for 25 seconds, into a 128 x 128 matrix with magnification of 1.33, resulting in a pixel size of 3.38 mm. For the INFINIA dual-head 3/8 HK4 camera a circular orbit over 360 degree, 180 degree arc for each detector was used, with the radius minimised for each patient by camera head autocontouring using collision detectors. The total number of projections was 120, each acquired for 25 seconds, with a 20% symmetrical energy window centred on the 140 keV photopeak; the matrix size was

128x128 and the applied zoom was 1.33, which resulted in a pixel size of 3.32 mm.

Images were checked for movement and reconstructed using a filtered back projection method with scatter correction. Further information on the reconstruction process for the cameras is detailed by Kemp et al (2005) (36). Recommended procedure guidelines for brain perfusion SPECT were followed and are described in detail by Juni et al (2009) and Kapucu et al (2009) (32,33). A routine protocol restricts patient use of medications that could potentially interfere with tracer binding prior to scanning.

Due to the clinical nature of the data obtained and used in this thesis, images were obtained on multiple cameras. SMV and Infinia cameras were however shown to be compatible by UHS medical physics quality control when a small sample of 9 patients and 5 printed subresolution sandwich phantoms as per methods detailed in Holmes et al (2013) (205) were imaged on both cameras (unpublished data, Tossici-Bolt et al 2017).

2.4.2.1.1 Statistical parametric mapping 8 pre-processing

In preparation for statistical analysis, the HMPAO SPECT reconstructed images were spatially normalised and smoothed using statistical parametric mapping 8 (SPM8) (38). The image origins were first moved to be located on the anterior commissure for all images. Images were then normalised to an 8mm cerebellar scaled template image with SPM8 estimation and writing options kept at default. After normalisation, images were smoothed using a 16mm smoothing kernel and cerebellar count normalisation was completed using an in-house MATLAB code (36,108). The cerebellar count normalisation scales the images to the mean counts of a cerebellar region of interest consisting of two circles of five pixels in diameter which were placed on to the hottest part of each cerebellar hemisphere. The cerebellum region was used for normalisation as it has little perfusion loss in dementia in comparison to other reference regions, and cerebellum normalisation has been shown to be more sensitive than a global normalisation procedure (86). During the cerebellar normalisation, correction for age was applied to account for normal perfusion decline seen in natural aging. This was completed using an age factor produced from an exponential fit for natural decline derived from a control sample (controls used

are described below). Options used for SPM8 normalisation and smoothing can be found in the Appendices.

2.4.2.1.2 Region of interest analysis

To obtain dementia imaging patterns, different software packages were used to define specific regions of interest and to extract voxel values for each region defined. Regions were defined using WFU-pickatlas software (206,207) and the Automated Anatomical Labelling digital (AAL) atlas (208). The AAL is set in the Montreal Neurological Institute (MNI) space. The Raw non-scaled data (average voxel value counts) was then obtained for each region of interest using the SPM8 MarsBaR toolbox for both patients and controls (209). These average voxel counts give a representation of regional activity counts and therefore provides an indirect measure of perfusion for a given region of the brain.

2.4.2.1.3 EXINI software analysis

EXINI diagnostics is an automated 3D SSP software used to observe perfusion abnormalities in HMPAO SPECT imaging, with details described by Hagerstrom et al (210). In brief, the EXINI software method defines the surface shape of the brain and identifies the maximum counts 0-1.5cm deep, before projecting this value onto a surface point. The surface projection values are divided into the relative cortical lobes and are presented relative to the cerebellum, cerebellar maximum or the whole cortex. This automated method of hypoperfusion visualisation was used only as part of clinical care to aid image reporting.

2.4.2.2 DaTscan SPECT imaging

The DaTSCAN images were acquired on a GE (SMV) DST-XL dual-head or Mediso Nucline x-Ring/4HR gamma camera with low energy high resolution collimators, using a ^{123}I-FP-CIT tracer. A circular orbit was used and 128 projections were acquired producing a 128 x 128 matrix with a pixel size of 2.03 mm. OSEM reconstruction with 12 iterations and 8 subsets was completed, with a butterworth filter for 3D postfiltering.

DaTSCAN tracer uptake and binding was calculated for patients independently from (^{99m}Tc) HMPAO SPECT results using the specific binding ratio (SBR). Calculation of the SBR allows quantitative assessment of the dopaminergic function of the striatum in patients when compared to controls. The SBR is

calculated using the specific uptake of the radioactive tracer (to the striata) and the non-specific uptake of the tracer (tracer not bound to striatal dopamine transporter and free tracer). A geometrical volume of interest covering the striatum captures all counts related to striatal binding, and non-specific counts are measured from a large volume of interest covering the whole cortex except for the striatum and edge of the cortex. The SBR is then calculated as the ratio of concentration of specific to non-specific radioactivity, as described by Bolt et al (211).

As with HMPAO SPECT, patients were imaged while awake with eyes open in a darkened room. Additionally, a routine protocol restricts patient use of medications that could potentially interfere with tracer binding prior to scanning.

2.4.2.3 Imaging control data

Normal controls were used to identify areas of abnormal tracer uptake in both perfusion SPECT and DaTSCAN analysis. The control samples reported in the thesis are currently used as part of the clinical analysis procedure by the UHS Nuclear Medicine and Medical Physics and Bioengineering department.

2.4.2.3.1 HMPAO SPECT controls

A group of perfusion SPECT controls were used throughout this thesis, obtained on the SMV dual-headed camera (parameters described above). 31 individuals were scanned with an average age of 66.6 years old (Mean, S.D. = 10.43, range 40 to 83) and a male/female split of 16/15. Control data used was identified in prior papers by Kemp et al (36,212). Controls were identified to have no obvious evidence of cognitive impairment, gave no evidence of history of head injury, psychiatric or neurological disorder and did not have hypertension, heart disease or vascular risk factors. Image acquisition, reconstruction and processing was identical for patients and controls.

2.4.2.3.2 DaTSCAN controls

DaTSCAN controls were utilised from the [^{123}I]FP-CIT ENC-DAT normal database (213,214). In brief, the ENC-DAT normal database consists of 151 healthy controls (80 men, 71 women) with an average age of 53 years old (Mean, range 20 to 83) recruited in 13 different centres across Europe. All

control subjects were healthy according to medical history, neurological examination, blood chemistry and psychiatric evaluation. Subjects had no evidence of cognitive impairment as assessed by a mini-mental state examination (score ≥28). Images obtained through the ENC-DAT normal database were reconstructed and acquired in a manner compatible to the patient population (215).

2.4.3 Other biomarkers

2.4.3.1 Cerebrospinal fluid

Cerebrospinal fluid (CSF) Alzheimer's disease biomarkers results were collected from patients who underwent a lumbar puncture as part of their clinical care at UHS. The lumbar puncture was performed with the patient on their side in a 'tucked' position, with collection normally completed in the L3/4 or L4/5 interspace. At least 1 to 2ml of CSF was collected per patient in polypropylene tubes. Samples were centrifuged at 3000 rpm for 10 minutes and supernatant fluid transferred into a 1.5ml polypropylene tube. Samples were then stored at -80°C within one hour of being taken and sent on dry ice to the Neuroimmunology and CSF laboratory at the Institute of Neurology, University College London Hospital (UCLH) where CSF biomarker analysis was completed. The Institute of Neurology, UCLH is involved in the Alzheimer's Association external quality control program to ensure test reliability. Total tau (T-tau), amyloid beta$_{1-42}$ (Aβ42) and phosphorylated tau (P-tau) concentrations were obtained by enzyme-linked immunosorbent assay (ELISA) and the ratio of T-tau to Aβ42 was calculated from the resulting concentrations.

Normal clinical cut-off values at the time of analysis were:

Total tau: 146 – 595 pg/mL
Amyloid beta$_{1-42}$: 627 – 1322 pg/mL
Phosphorylated tau: 24 – 68 pg/mL
Total tau to Amyloid Beta$_{1-42}$ ratio: < 1.00

2.4.4 Statistical analysis

Statistical analysis throughout the thesis was completed using the Statistical Package for Social Sciences software (SPSS v22).

Chapter 3: DaTSCAN biomarker pattern validation

3.1 Introduction

^{123}I-FP-CIT single photon emission computed tomography (DaTSCAN) imaging is an established biomarker for antemortem diagnosis in DLB, and is included in the current consensus diagnostic criteria (28). It has good sensitivity and specificity for distinguishing DLB from AD, and other forms of dementia compared with both clinical and neuropathologically validated diagnosis (216–218). In clinical practice when diagnosing dementia, it is often requested in conjunction with functional imaging techniques such as perfusion single photon emission computed tomography (SPECT) imaging, which are more useful at distinguishing non-DLB dementia subtypes.

As discussed in Chapter 1, early DLB and AD patients often show similar patterns of global cerebral perfusion, making differentiation between the subtypes difficult when the clinical presentation is also similar (72). The presence of mixed pathology may also confound diagnosis in some cases (219). Previous studies have shown that regional analysis of occipital hypoperfusion, particularly of the medial region, on HMPAO SPECT may be a specific biomarker for DLB, however its reliability in clinical settings is debated (72,120,212,220,221). DLB patients may also have relatively preserved function of the posterior cingulate in comparison to AD (the "posterior cingulate island sign"), where significant functional deficits are seen (125,126). The clinical utility of perfusion SPECT in identifying posterior cingulate preservation for DLB diagnosis is however also a point of contention (97). The combination of occipital regional functional deficits with preservation of posterior cingulate function could be a useful biomarker to distinguish DLB from AD and reduce the need for further DaTSCAN imaging where functional imaging has already been completed (222).

Although previous studies have investigated the accuracy of perfusion SPECT and DaTSCAN imaging to distinguish DLB from AD, to our knowledge none have explored the use of quantitative region of interest analysis on perfusion SPECT imaging to predict DaTSCAN results in a mixed clinical sample. We

aimed to investigate the extent to which *a priori* occipital and posterior cingulate regions of interests on HMPAO SPECT may be diagnostically specific for DLB in patients with abnormal DaTSCAN imaging. We hypothesised that the combination of these regions on HMPAO SPECT could accurately predict an individual's DaTSCAN status, and therefore aid DLB diagnosis.

3.2 Hypothesis

1. The combination of occipital lobe hypoperfusion and posterior cingulate preservation can be used to identify individuals with abnormal DaTSCAN imaging in a mixed clinical sample.

3.3 Contributions and Collaborations

The study was conceived and designed by the author and Dr C. Kipps. Participant recruitment was via retrospective review of clinical files of patients undergoing diagnostic testing in clinics held by Dr C. Kipps, Dr G. Pengas and Dr B. Ghosh. Ethical approval for these studies (RetroBRAIID) was obtained by the author. Initial image processing for both HMPAO SPECT and DaTSCAN imaging was performed by Dr L. Tossici-Bolt, Dr S. Michopoulou and Mr E. Varzarkis. All subsequent data processing and statistical analysis with DaTSCAN biomarker results was performed by the author. Interpretation of the data and preparation of tables and figures was carried out by the author.

3.4 Methods

3.4.1 Sample

Ninety-nine patients with cognitive complaints who received both (^{99m}Tc) HMPAO SPECT and DaTSCAN imaging in a dementia diagnostic centre (University Hospital Southampton) as part of their clinical care were retrospectively identified. Images were obtained as per the NHS Health

Research Authority approved Retrospective Brain Imaging in Dementia study (RetroBraIID, see Chapter 2). Patients were not selected by diagnosis to ensure results were applicable to a memory clinic setting, and diagnostic doubt existed for all patients at the time of scanning. The average time between HMPAO SPECT and DaTSCAN acquisition was 105 days. Thirty-one HMPAO SPECT controls were obtained for analysis (SMV / INFINIA camera controls). HMPAO control individuals did not have DaTSCAN imaging completed.

3.4.2 DaTscan specific binding ratio quantification

DaTscan SPECT images were collected, reconstructed and processed as per the University Hospital Southampton NHS Foundation Trust procedures outlined in Chapter 2 (Methods). DaTscan tracer uptake and binding was objectively obtained independently from (^{99m}Tc) HMPAO SPECT results using the specific binding ratio (SBR) for both patients and controls.

3.4.3 Perfusion SPECT imaging regions of interest

Perfusion SPECT images were collected, reconstructed and processed as per the University Hospital Southampton NHS Foundation Trust procedures outlined in Chapter 2. All images used were completed on a GE (SMV) DST-XL dual-head gamma camera which had low-energy, high resolution collimators.

A priori regions of interest (ROI) for the posterior cingulate, precuneus, medial occipital lobes and lateral occipital lobes were created for each hemisphere using WFU-pickatlas software (206,207) and the Automated Anatomical Labelling digital (AAL) atlas (208), see Figure 3-1. Average voxel value counts were then obtained for each region of interest using the SPM8 MarsBaR toolbox for both patients and controls (209).

3.4.4 Statistical analysis

Individuals were grouped into abnormal and normal DaTSCAN groups based on an age-dependent recommended cut-off 2 standard deviations away from the [^{123}I]FP-CIT ENC-DAT normal database mean (213,214). DaTSCAN controls from the ENC-DAT normal database from which the cut-offs were derived were reconstructed and acquired in a manner compatible to the patient population (215).

Voxel based t-maps of the HMPAO scans comparing normal and abnormal DaTSCAN groups to each other and controls were produced using SPM8. SPM8 maps were extracted at a $p < .05$ family wise error (FWE) corrected significance with 100 cluster voxel threshold. (^{99m}Tc) HMPAO SPECT perfusion values for (1) individual ROIs, (2) combined occipital and posterior cingulate, and (3) combined posterior cingulate and precuneus ROIs, were compared between the DaTSCAN abnormal and normal groups using t-tests.

Finally, patients were grouped based on posterior cingulate, medial and/or lateral occipital, precuneus and cuneus perfusion both individually and in combination. We designated posterior cingulate preservation in combination with medial, lateral or whole occipital lobe hypoperfusion as PCing-MedOcc+, PCing-LatOcc+, and PCing-AllOcc+ respectively. We designated posterior cingulate preservation with precuneus hypoperfusion as PCing-Prec+, and posterior cingulate with precuneus and cuneus hypoperfusion as PCing-PrecCuneus+. Preserved posterior cingulate function was defined as an average perfusion voxel value less than two standard deviations away from control mean.

Cross-tabulation was used to evaluate the predictive power of posterior cingulate preservation, occipital lobe hypoperfusion and precuneus hypoperfusion both individually and combined in identifying DLB disease as defined by a positive (abnormal) DaTSCAN.

Table 3-1 Sample demographics

Abnormal DaTSCAN was defined as a specific binding ratio of more than 2 standard deviations (S.D.) away from the [123I]FP-CIT ENC-DAT normal database mean (213). HMPAO SPECT DLB type was defined as an abnormal medial and lateral occipital perfusion (> 2 S.D. below control mean) combined with a normal posterior cingulate cortex perfusion (< 2 S.D. below control mean). Abbreviations: PCing, Posterior cingulate.

	DaTscan normal	DaTscan abnormal	SPECT DLB type	SPECT AD type	Statistics (*p* value)
Patients (Male)	50 (30)	49 (28)	12 (8)	87 (50)	-
Age in years (mean ± S.D.)	72.7 ± 10.1	71.9 ± 7.5	71.9 ± 11.1	72.4 ± 8.6	*ns*
Months between HMPAO SPECT and DaTSCAN (mean ± S.D.)	3.1 ± 4.8	3.2 ± 4.8	3.2 ± 4.6	2.9 ± 4.0	*ns*

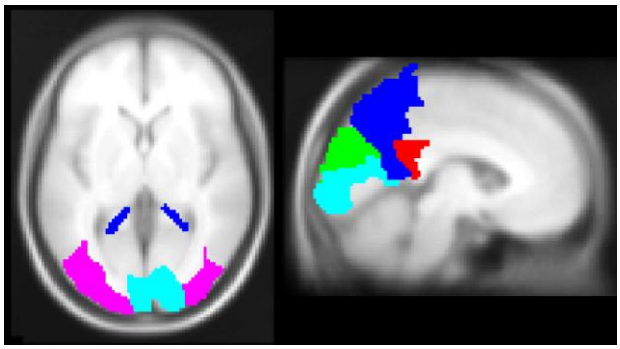


Figure 3-1 Regions of interest used in the analysis

The regions of interest (ROIs) used in the analysis on axial and coronal slice view. The lateral occipital (violet), medial occipital / calcarine fissure (turquoise), posterior cingulate (red), cuneus (green) and precuneus (blue) is shown. The ROIs were obtained from the automatic anatomical labelling (aal) atlas, based in Montreal Neurological Institute (MNI) space.

3.5 Results

3.5.1 Group differences

Demographics of the sample can be seen in Table 3-1. When compared to controls using statistical parametric mapping, both normal (DaTSCAN negative) and abnormal (DaTSCAN positive) groups showed significant perfusion deficits with very similar regional abnormalities ($p < .05$ FWE corrected). The main areas of hypoperfusion included parietal, parietotemporal and occipital lobes, with further hypoperfusion in the superior frontal lobe, and superior posterior temporal lobe compared to controls. When perfusion deficits against controls for both groups were overlaid, deficits were more extensive in the abnormal DaTSCAN group, with extended hypoperfusion in the superior frontal lobe, medial prefrontal cortex and a region adjacent to the posterior cingulate (Figure 3-2). However, direct comparison of DaTSCAN groups showed no significant clusters at $p < .05$ FWE corrected threshold. No significant perfusion differences were found between DaTSCAN groups in any of the *a priori* ROIs defined when mean perfusion voxel values were compared using t-tests ($p > .05$).

3.5.2 Cross-tabulation

The combined *a priori* ROI DLB pattern of normal posterior cingulate perfusion with abnormal medial and lateral occipital perfusion (PCing-AllOcc+ group) showed very high specificity and positive prediction values for the presence of abnormal DaTSCAN (98% and 92% respectively), with a positive likelihood ratio of 11.1. Sensitivity and negative predictive values were however low (22% and 56% respectively). A Pearson Chi-square test of independence showed a significant relationship between DaTSCAN and HMPAO results, χ^2 (1, N = 99) = 9.72, p = .002.

When the medial and lateral occipital regions were examined individually with the posterior cingulate (PCing-MedOcc+ and PCing-LatOcc+ groups), the medial occipital region was more predictive than the lateral occipital region. PCing-MedOcc+ specificity and positive predictive value was only slightly reduced from the combined occipital regions (96% and 85%) and sensitivity and

negative predictive values were similar (22% and 56%), with an overall positive likelihood ratio of 5.6 (Table 3-2).

Classifying patients using the precuneus to posterior cingulate perfusion ratio (PCing-Prec+ group) had high sensitivity, but very poor specificity of 14% and a positive and negative likelihood ratio of 1.0. Including the cuneus with the cingulate island sign and precuneus (PCing-PrecCuneus+) did not increase prediction accuracy. No individual regional biomarker could accurately predict DaTSCAN result.

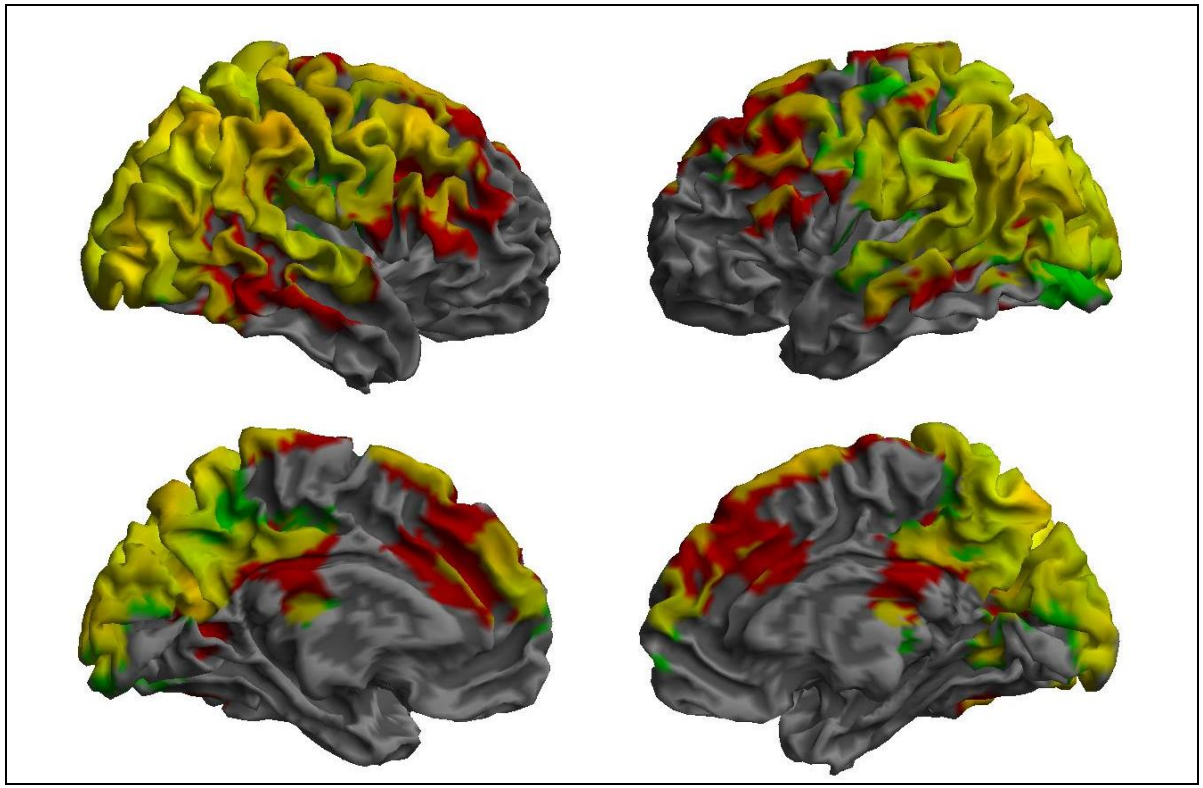


Figure 3-2 SPM comparisons of DaTSCAN groups against controls

Areas of hypoperfusion on HMPAO SPECT in DaTSCAN positive (red) and DaTSCAN negative (green) individuals when compared to controls. Areas of yellow indicate areas where hypoperfusion is present in both groups. $p < .05$ FWE corrected with 100 voxel cluster threshold.

Table 3-2 Accuracy of individual and combined regions in predicting DaTSCAN status

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios (LR) for individual and combined regions of interest in predicting DaTSCAN result.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR
Medial occipital	39	60	49	50	0.969	1.02
Lateral occipital	61	36	48	49	0.957	1.08
Posterior cingulate	76	40	55	63	1.26	0.612
Precuneus	45	50	47	48	0.898	1.10
PCing-MedOcc+	22	96	85	56	5.61	0.808
PCing-LatOcc+	37	76	60	55	1.53	0.832
PCing-AllOcc+	22	98	92	56	11.2	0.791
PCing-Prec+	86	14	49	50	1.00	1.02
PCing-PrecCuneus+	92	4	48	33	0.957	2.04

3.6 Discussion

This study aimed to elucidate the usefulness of occipital lobe and posterior cingulate gyrus perfusion on HMPAO SPECT in distinguishing DLB type dementia in diagnostically ambiguous clinical samples using nigrostriatal dopamine transporter imaging validation as gold standard. Our results show that the combination of reduced occipital lobe perfusion combined with preserved posterior cingulate perfusion (PCing-AllOcc+) in an individual is highly specific for DLB, despite visually near-identical group perfusion patterns in cognitively impaired individuals with abnormal, compared to normal, DaTSCANs. A high positive likelihood ratio highlights the potential clinical utility of this regional combination on HMPAO SPECT imaging to rule-in DLB.

The findings presented in this study highlight both the potential clinical use and the limitations of occipital lobe and posterior cingulate cortex ROI analysis on perfusion SPECT imaging for aiding DLB diagnosis. When compared to controls by statistical parametric mapping, both abnormal and normal DaTSCAN groups showed significantly reduced perfusion ($p < .05$ FWE corrected) across the cortex (Figure 3-2), with slight differences in superior frontal and medial regions only clearly visible upon direct overlay of the SPM maps. The similarity in hypoperfusion between the groups highlights the previously described difficulty for visual distinction between DLB and AD groups, particularly in clinical samples where mixed pathologies can exist (36).

Interestingly, direct voxel based comparisons of the DaTSCAN groups at standard corrected thresholds ($p < .05$ FWE) did not highlight hypoperfusion in the occipital lobe for the DLB group over non-DLB as previously shown in multiple studies (72,223–225), nor did they identify posterior cingulate preservation (86). Comparison of the mean perfusion voxel values also found no difference between groups in either individual or combined ratio ROIs. This may be due to these biomarkers being present in some, but not all, DLB patients, with group comparisons masking abnormality in some individuals. This is consistent with studies that have found both occipital lobe hypoperfusion and preservation of the posterior cingulate gyrus to be unreliable clinical biomarkers for DLB on perfusion SPECT imaging (84,97,212,226).

Despite the absence of significant DaTSCAN group differences in any of the a *priori* regions, the combination of lateral and medial occipital lobe hypoperfusion with normal posterior cingulate perfusion (PCing-AllOcc+) distinguished individuals with abnormal from normal DaTSCAN imaging with a high level of specificity in our unselected clinical sample. The high positive likelihood ratio of 11.1 suggests that the use of individual perfusion values to identify those with this pattern of perfusion could be a useful tool for aiding clinicians in the diagnosis of DLB when the clinical presentation is ambiguous for a dementia subtype. The medial occipital lobe was found to drive the predictor accuracy, a finding consistent with other studies that suggest that medial occipital lobe perfusion is preferentially reduced in DLB (227,228). The sensitivity and negative predictive value of the combined regions (PCing-AllOcc+) was however poor, and the classifier could not accurately predict a normal DaTSCAN result when the PCing-AllOcc+ regional combination was not present. We also found the combination of posterior cingulate to precuneus and cuneus ratio (PCing-Prec+ and PCing-PrecCuneus+) was ineffective in predicting DaTSCAN profiles in our sample, despite previous functional imaging studies suggesting their usefulness in the prediction of DLB over AD (125,222).

A recent study by Imabayashi et al (2016) using technetium-99m ethyl cysteinate dimer (ECD) SPECT and z-score analysis in 17 AD and 18 probable DLB individuals found that medial occipital lobe hypoperfusion in combination with the cingulate island sign gave a high receiver operator characteristic curve area under the curve of 0.87 when classifying DLB individuals from amyloid positive AD patients (222). This was subsequently validated in another small sample study (229). Although our findings do agree with the conclusion that the combination of these regions can be useful in clinical diagnosis of DLB, we found a much lower sensitivity in our sample (22% compared to 89%), suggesting the regions are not useful for predicting normal DaTSCAN (or non-DLB) individuals.

The difference in accuracy found is likely due to differing samples and classification of disease groups. Our large sample of individuals with diagnostic doubt were classified using DaTSCAN imaging, rather than classifying probable DLB individuals by clinical diagnosis alone. Additionally, we compared these DaTSCAN validated DLB individuals to any non-DLB

diagnosis as diagnosed by a negative DaTSCAN, rather than amyloid validated AD only. A 2015 Cochrane review on the use of DaTSCAN imaging for classification of DLB concluded the technology was more accurate than consensus clinical criteria in discriminating DLB from AD (230).

Although we did not attempt to classify individuals with a negative DaTSCAN in our sample, the ability to predict an individual's DaTSCAN status is directly useful for dementia clinicians in DLB diagnosis when diagnostic doubt exists. Our use of a naturalistic clinical sample should ensure results demonstrate the ROIs clinical utility. Results from this study highlight the potential usefulness of a quantitative tool that identifies regional hypoperfusion of the occipital and preservation of the posterior cingulate regions in aiding clinician identification of DLB patients when visual rCBF patterns are ambiguous.

The conflicting literature that exists on the usefulness of perfusion SPECT imaging in DLB diagnosis may be due to an inherent lack of resolution in the technology. Although semi-quantitative and quantitative analysis techniques such as SPM have been shown to improve diagnostic accuracy of HMPAO SPECT over visual assessment alone, FDG PET studies show consistently better sensitivity and specificity values for diagnosis of DLB with the use of occipital and/or posterior cingulate regions of interest (97,123). The use of a small and highly defined region of interest such as the posterior cingulate of the aal atlas may not be optimal for HMPAO SPECT, reducing accuracy for discrimination between DLB and other dementias.

There are several limitations to this study. The sample used was collected retrospectively from a clinical cohort, therefore long-term follow-up or histopathology to confirm diagnosis post imaging was not obtained. Although DaTSCAN imaging is widely regarded as the gold standard for antemortem DLB diagnosis, it is not 100% accurate, with an estimated 100% specificity and 88% sensitivity when assessed semi-quantitatively, as in this study, and validated with autopsy results (218). We note however, that clinical validation is less accurate than DaTSCAN imaging in diagnosis (230). Research into individuals with conflicting clinical profiles and DaTSCAN results are limited, however some individuals with atypical forms of DLB have been shown to have normal DaTSCAN imaging (231,232). We were unable to ascertain whether therapeutic medications were prescribed in the interval between the HMPAO SPECT and DaTSCAN. We do not believe this is likely to have a bearing on our results as

medications usually prescribed in this situation, namely L-Dopa, dopamine agonists and cholinesterases are not believed to interfere with DaTSCAN binding (233–235). Finally, as with many imaging biomarkers, there is no consensus on the ideal cut-off value for either HMPAO SPECT perfusion values or the striatal binding ratio on DaTSCAN, and therefore 2 standard deviations away from controls may not be optimal for identification of DLB.

In conclusion, our findings show that the combination of medial and lateral occipital hypoperfusion with preserved posterior cingulate gyrus perfusion is highly specific for individuals with a positive DaTSCAN in a clinical sample where diagnostic doubt exists. This regional combination however lacks sensitivity and is present only in some DLB individuals, therefore absence of the sign cannot be used to rule out DLB. A positive finding provides strong evidence to rule in DLB.

Chapter 4: CSF biomarker pattern validation

4.1 Introduction

Alzheimer's disease cerebrospinal fluid (CSF) biomarkers show high sensitivity and specificity for AD detection and are included in AD diagnostic criteria (18,47–49). Analysis of CSF amyloid and tau levels offers the ability to measure protein concentration changes that reflect pathology seen in the disease, specifically the formation of neuritic plaques and neurofibrillary tangles in the brain (46). A decrease in amyloid-β42 (Aβ42) in CSF denotes an increase in deposition of the peptide into Alzheimer's disease related plaques, and an increase in phosphorylated tau (P-tau) is indicative of the pathological release of tau from microtubules and the formation of intracellular tangles (236–238). An increase in CSF total tau (T-tau) concentration provides a marker of general neuronal damage and neurodegeneration, with higher levels associated with a faster decline and disease progression (239,240).

Biomarker interpretation is not always straightforward however and a range of factors may influence the ability to use CSF biomarkers to determine likelihood of underlying Alzheimer's disease pathology. A study by the Paris-North, Lille and Montpellier (PLM) group has shown a simple combined scale rating patients by the number of abnormal CSF biomarkers (amyloid beta 42, total tau and phosphorylated tau protein levels) using recommended reference ranges, can predict likelihood of AD pathology (241). The scale provides a predictive risk of AD, with 0, 1, 2 and 3 abnormal biomarkers (PLM 0, 1, 2 and 3 respectively) indicating a 10%, 25%, 77% and 94% chance of AD respectively (see Table 4-1).

CSF biomarkers are however not widely available for clinical use in the diagnosis of AD and require an invasive test (lumbar puncture) to obtain the fluid for analysis. Additionally, they have less use in aiding identification of non-AD subtypes of dementia. For these reasons, CSF is commonly completed secondary to clinical interview and imaging workup. Improved characterisation of imaging patterns on HMPAO SPECT imaging predictive of underlying Alzheimer's disease as defined by CSF biomarker status would be useful and could aid earlier confident diagnostic reporting, particularly where CSF analysis is not easily available.

The relationship between CSF AD biomarkers and perfusion SPECT (rCBF) imaging has previously been investigated in both AD (93,165,172,242) and non-AD (88,138,139) dementia, with conflicting results. A long-term longitudinal study of MCI individuals found a significant association between CSF biomarker profiles and extent of parietal hypoperfusion, with a larger parietal deficit related to lower Aβ42 and higher T-tau and P-tau levels (165). The left parietal region, particularly the inferior parietal gyrus and precuneus, and temporoparietal regions have also been shown to be related to AD type CSF biomarkers (88,138,139,172,243). Other studies have however found no correlation between rCBF and CSF (93,242).

Prior studies investigating rCBF and CSF AD biomarkers often use selected samples that do not represent the clinical population and rarely investigate imaging patterns seen in the individual, limiting their practical utility in diagnostic reporting. This study aimed to validate perfusion SPECT imaging patterns by identifying regions on perfusion SPECT imaging that could best predict combined CSF biomarker status using the PLM scale and therefore overall AD likelihood. Additionally, we aimed to explore what these patterns look like in the individual.

Table 4-1 Paris-North, Lille and Montpellier (PLM) cerebrospinal fluid predictive scale

Likelihood of Alzheimer's disease (percentages) when classifying patients based on the PLM scale (241).

CSF Amyloid Beta1-42, total tau, and phosphorylated tau			
0 pathologic biomarkers	1 pathologic biomarker	2 pathologic biomarkers	3 pathologic biomarkers
PLM 0	**PLM 1**	**PLM 2**	**PLM 3**
<10%	<25%	>75%	>90%

4.2 Hypothesis

1. HMPAO SPECT can accurately predict individuals with Alzheimer's disease as defined by combined CSF AD biomarker results (Aβ42, T-tau, P-tau).
2. Individual statistical parametric mapping (SPM) hypoperfusion patterns on HMPAO SPECT scans correlate with underlying Alzheimer's disease likelihood (PLM scale).

4.3 Contributions and collaborations

The study was conceived and designed by the author and Dr C. Kipps. Participant recruitment was via retrospective review of clinical files of patients undergoing diagnostic testing in clinics held by Dr C. Kipps, Dr G. Pengas and Dr B. Ghosh. Ethical approval for this study (RetroBrainID) was obtained by the author. Initial image processing was performed by Dr L. Tossici-Bolt, Dr S. Michopoulou and Mr E. Varzarkis. All subsequent data processing and correlation of results with CSF biomarker results was performed by the author. Interpretation of the data and preparation of tables and figures was carried out by the author.

4.4 Methods

4.4.1 Sample

A series of 69 diagnostically ambiguous subjects with HMPAO SPECT brain imaging and AD cerebrospinal fluid biomarkers was analysed. One subject was removed from the analysis due to their very young age (25 years old), leaving a sample size of 68. P-tau concentrations were not available for 7 subjects, with five of these individuals unable to be classified into PLM group (further details described below). If individuals had multiple HMPAO SPECT scans, the scan closest to the time of CSF acquisition was used. The average time between HMPAO SPECT and CSF acquisition was 99 days (S.D. 214.5). Demographic features of the cohort can be seen in Table 4-2.

4.4.2 Data collection and analysis

Data used was collected under the NHS Health Research Authority approved Retrospective Brain Imaging in Dementia study (RetroBrainID; protocol in Appendix, further details in Chapter 2).

Images were acquired and reconstructed as per the University Hospital Southampton NHS Foundation Trust clinical protocol detailed in Chapter 2 (Methods). Images were obtained on either the GE (SMV) DST-XL or INFINIA dual-headed gamma cameras. Once reconstructed, images were normalised and smoothed using SPM8 and cerebellar count normalised with age correction using an in house matlab code as described in Chapter 2. Regional perfusion measures for 23 regions across the brain were extracted using the Automated Anatomical Labelling atlas (AAL) in conjunction with WFU-pickatlas and MarsBaR software (206–209). Exact ROIs are shown in Table 4-3. Cerebrospinal fluid was collected during clinical diagnostic procedures and analysed as per the protocol described in Chapter 2 (Methods). CSF Aβ42, T-tau and P-tau protein concentrations and tau to Aβ42 ratios were obtained. Amyloid beta 1-42 < 627 pg/ml, total tau > 595 pg/ml, phosphorylated tau > 68 pg/ml and tau to amyloid ratio > 1.0 were classified as abnormal, based on accepted reference ranges at the time of the study. CSF biomarker results for the cohort can be seen in Table 4-4.

Table 4-2 Sample demographics

	PLM 0	PLM 1	PLM 2/3	Statistics (*p* value)
Patients (M/F)	17/9	9/7	10/11	*ns*
Age in years (mean ± S.D.)	59.7 ± 7.05	59.1 ± 5.95	57.9 ± 5.84	*ns*
Days between scan and CSF (mean ± S.D.)	50.2 ± 155.7	126.3 ± 290.7	146.6 ± 226.3	*ns*

* Five patients who did not have P-tau values and were unable to be grouped are not included in the table data, however did not significantly differ in gender, age, days between scan and CSF or camera used from PLM 0, 1, or 2/3 groups.

Table 4-3 Regions of interest obtained for use in discriminant analysis

Left and right values were collected separately for all regions except the caudate and putamen and posterior cingulate, where a combined bilateral value was used.

Frontal	Temporal	Parietal	Occipital	Other
Superior gyrus	Superior gyrus	Superior gyrus	Superior gyrus	Caudate and putamen
Middle gyrus	Middle gyrus	Inferior gyrus	Middle gyrus	Posterior cingulate
Inferior gyrus	Inferior gyrus	Supramarginal gyrus	Inferior gyrus	
Orbitofrontal	Superior pole	Angular gyrus	Cuneus	
Insula	Middle pole	Precuneus		
Supplementary motor area	Medial temporal			

4.4.3 Statistical analysis

CSF biomarkers were first analysed using the PLM combined predictive scale of AD likelihood to identify patterns on imaging that best indicated underlying Alzheimer's type dementia. Discriminant analysis was then used to classify individuals into AD or non-AD groups based on region of interest analysis. Individual patient deficits and their appearance were then explored.

4.4.3.1 Patterns at group level and their predictive value for AD pathology

Patients were grouped based on the combined PLM CSF AD biomarker scale (Table 4-1) with 0, 1, 2 and 3 abnormal biomarkers classified PLM 0, 1, 2 and 3 respectively. Due to the small number of subjects with 2 abnormal biomarkers (N = 4 excluding those with missing p-tau values) these individuals were combined with subjects with 3 abnormal biomarkers on the basis that this group in previous research showed a relatively high risk of underlying AD. Two individuals without P-tau results but both T-tau and Aβ42 abnormal were also classified into the high risk PLM 2/3 group. Five other individuals without P-tau levels (all with normal T-tau and Aβ42) were included only in combined PLM group analyses where suitable. For example, those with normal T-tau and Aβ42 but P-tau results unavailable could be classified into a combined PLM 0 or 1 group but could not be classified into the PLM 0 only or PLM 1 only groups in individual analyses.

T-test analyses using statistical parametric mapping (SPM8) were performed to identify brain regions that best characterised group differences between increasing levels on the PLM scale. Three analyses were performed:

- The imaging from each PLM group (0,1, combined group 2/3) was compared with images from a control group
- PLM groups were compared with each other (0,1, combined group 2/3)
- A low risk group (combined PLM groups 0/1) was compared with a high risk group (combined PLM groups 2/3)

In a second analysis, preselected regions of interest from across the cerebrum known to be affected in dementia were used in a leave one out stepwise discriminant analysis to identify regions on the perfusion SPECT imaging that best predicted CSF PLM group status.

4.4.3.2 Patterns in the individual patient

In order to visualise patterns in the individual, single-subject voxel based perfusion maps were created for each patient in comparison to a control group (see Chapter 2 for further details) using statistical parametric mapping (SPM8). Images were thresholded at a $p < .001$ (uncorrected) with 100 voxel cluster threshold and displayed on glass brain and canonical structural images. This threshold was chosen as it was used in HMPAO clinical reporting at UHS at the time of writing. Subsequently, images were binarized using the threshold and overlaid to create perfusion heat maps for each PLM group (0, 1, 2/3) in order to identify which areas were most frequently abnormal at a single subject level. The colour scale was standardised based on the number of individuals in each group and the overlap heat maps were displayed using MRIcro image visualisation software (244).

4.5 Results

4.5.1 Combined CSF biomarkers

4.5.1.1 PLM groups against controls

When compared to controls using SPM8, PLM groups 0, 1 and 2/3 all showed significant regional hypoperfusion, with extent of the perfusion deficit increasing with the number of abnormal biomarkers (Figure 4-1, Figure 4-2, Figure 4-3). PLM 0 showed a small but significant region of hypoperfusion in the left occipital lobe at $p < .001$ FWE significance level when compared to controls. PLM 1 showed hypoperfusion of the left posterior cerebrum, particularly of the inferior parietal and occipital lobe at $p < .001$ FWE significance level. When this threshold was reduced to $p < .05$ FWE, widening hypoperfusion extent in the parietal and occipital lobes was seen, with right parietal involvement, in addition to left temporal and superior frontal regional hypoperfusion (particularly in the supplementary motor area) when compared to controls. Hypoperfusion for this group was predominantly left sided.

The PLM 2 or 3 group showed the most extensive reduction in perfusion when compared to controls, occurring bilaterally, with posterior predominance,

being confluent over the parietal and occipital lobes at $p < .001$ FWE. With reduction of the threshold to $p < .05$ FWE, posterior hypoperfusion extent widened, and a small region of frontal hypoperfusion can be also seen around the supplementary motor area. Note that the use of $p < .001$ FWE is a more stringent threshold than would normally be used in clinical practice, however it was used here to ensure that the regions of highest perfusion loss, and therefore those most useful in distinguishing the groups, could be detected.

4.5.1.2 Differences between PLM groups

When PLM groups 1 and 2/3 were compared against patients with no abnormal AD CSF biomarkers (PLM 0), both PLM1 and PLM 2/3 showed significantly worse perfusion in the left temporo-parietal region at a $p < .05$ FWE threshold (Figure 4-4). PLM 2/3 also showed relatively reduced perfusion in medial parietal regions.

Comparison of the low AD risk groups (combined PLM 0/1) against the high AD risk groups (combined PLM 2/3) identified small areas of hypoperfusion in the inferior parietal, medial parietal, posterior temporal and inferior occipital/superior cerebellum regions in the higher risk group. When the low AD risk groups (PLM 0/1) were compared against the highest risk AD group (PLM 3), a larger region of significantly lower perfusion was found predominantly in the inferior parietal angular gyrus and precuneus regions (Figure 4-5). Small areas of reduced perfusion were also seen in the posterior temporal lobe.

Table 4-4 Cerebrospinal fluid biomarker features of PLM groups

CSF biomarker levels for PLM 0, PLM 1 and PLM 2/3 groups are shown (mean ± standard deviation). 95% upper and lower confidence intervals for means are also shown in brackets. Five patients without p-tau values were unable to be grouped and are not included in the table data, however did not significantly differ in any CSF biomarker concentration from any PLM group.

	PLM 0	PLM 1	PLM 2/3
aβ-42 concentration (pg/ml)	1068.3 ± 243.6 (969.9 – 1166.7)	636.6 ± 373.7 (437.5 – 835.8)	412.6 ± 114.0 (357.7 – 467.5)
T-tau concentration (pg/ml)	247.4 ± 97.0 (208.2 – 286.6)	396.0 ± 191.6 (293.0 – 498.1)	1271.0 ± 875.2 (849.1 – 1692.8)
P-tau concentration (pg/ml)	40.1 ± 11.9 (35.3 – 44.9)	45.5 ± 17.2 (36.3 – 54.7)	108.5 ± 37.5 (90.4 – 126.5)
T-tau / aβ-42 ratio	0.23 ± 0.10 (0.19 – 0.27)	0.69 ± 0.31 (0.52 – 0.85)	3.18 ± 2.35 (2.05 – 4.32)

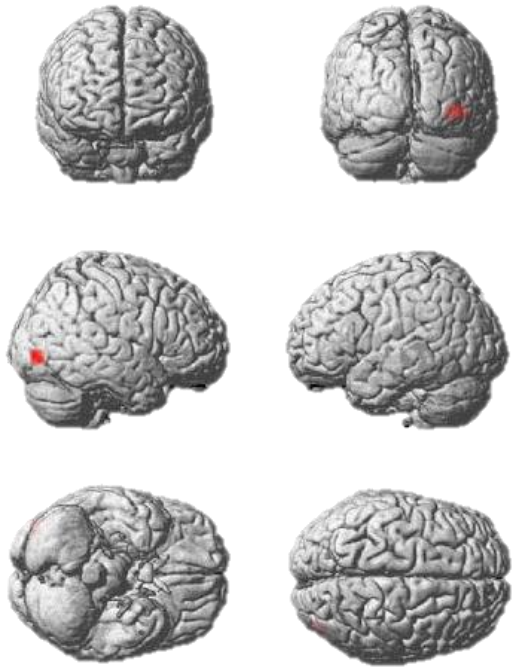
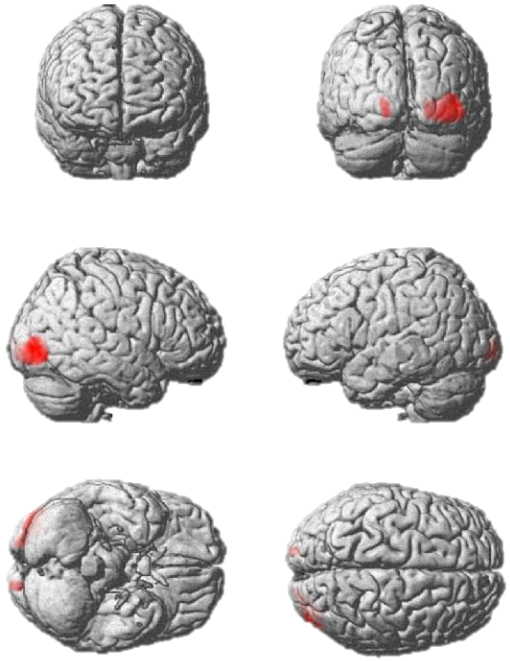
$p < .001$ FWE	$p < .05$ FWE
	

Figure 4-1 SPM comparisons of PLM group 0 and controls

Figures are shown at both $p < .001$ FWE and $p < .05$ FWE to show both extent of hypoperfusion and location of most significant regional abnormality. Areas of relative hypoperfusion in PLM group compared to controls are shown in red.

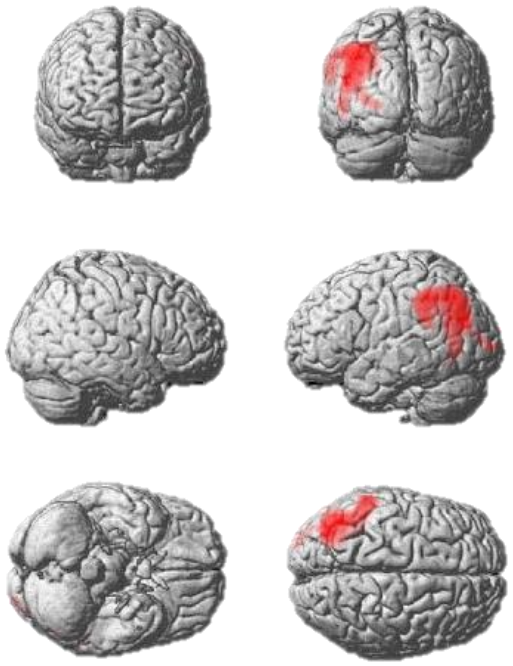
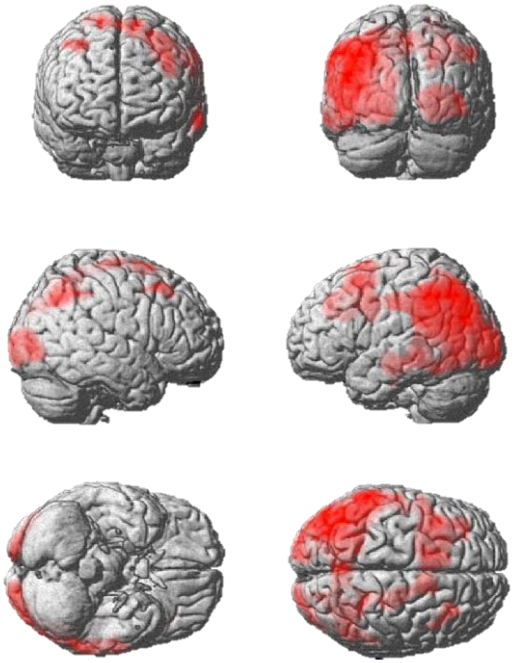
$p < .001$ FWE	$p < .05$ FWE
	

Figure 4-2 SPM comparisons of PLM group 1 and controls

Figures are shown at both $p < .001$ FWE and $p < .05$ FWE to show both extent of hypoperfusion and location of most significant regional abnormality. Areas of relative hypoperfusion in PLM group compared to controls are shown in red.

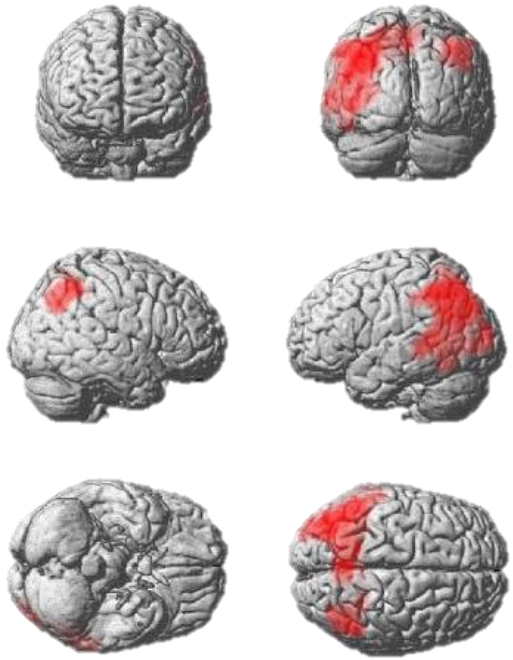
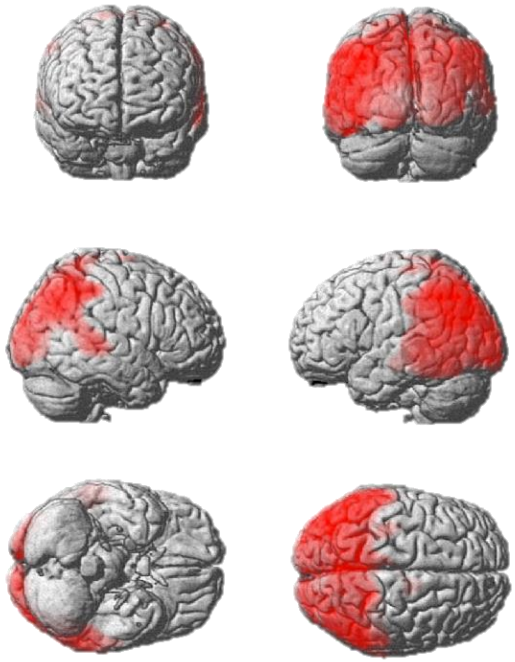
$p < .001$ FWE	$p < .05$ FWE
	

Figure 4-3 SPM comparisons of PLM group 2/3 and controls

Figures are shown at both $p < .001$ FWE and $p < .05$ FWE to show both extent of hypoperfusion and location of most significant regional abnormality. Areas of relative hypoperfusion in PLM group compared to controls are shown in red.

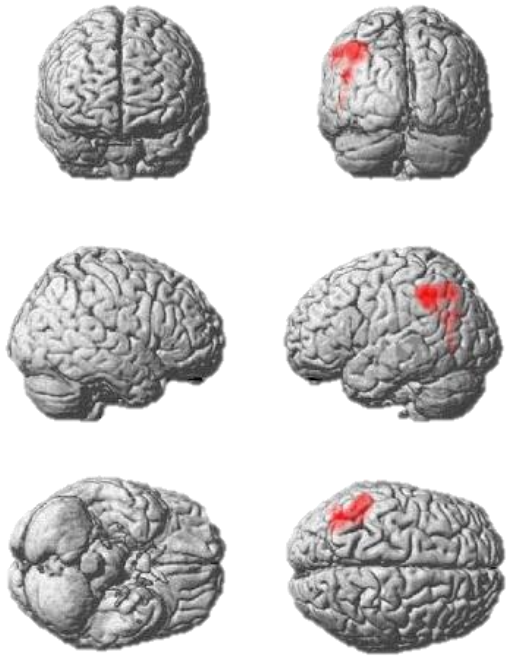
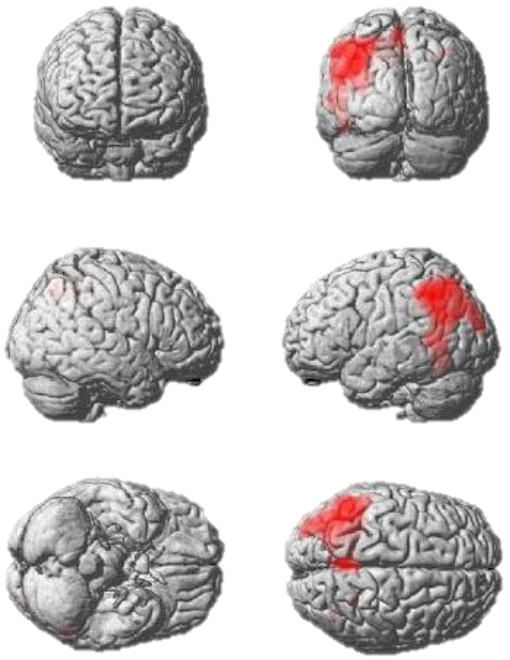
PLM 1	PLM 2 or 3
	

Figure 4-4 SPM comparisons of PLM group against group

Statistical parametric mapping (SPM) comparison showing areas of relative hypoperfusion (red) in low (PLM 1, Left) and high (PLM 2 and 3, Right) AD risk groups when compared to patients with normal CSF biomarkers (PLM 0). Results are shown at $p < .05$ FWE corrected with a 100 voxel cluster threshold.

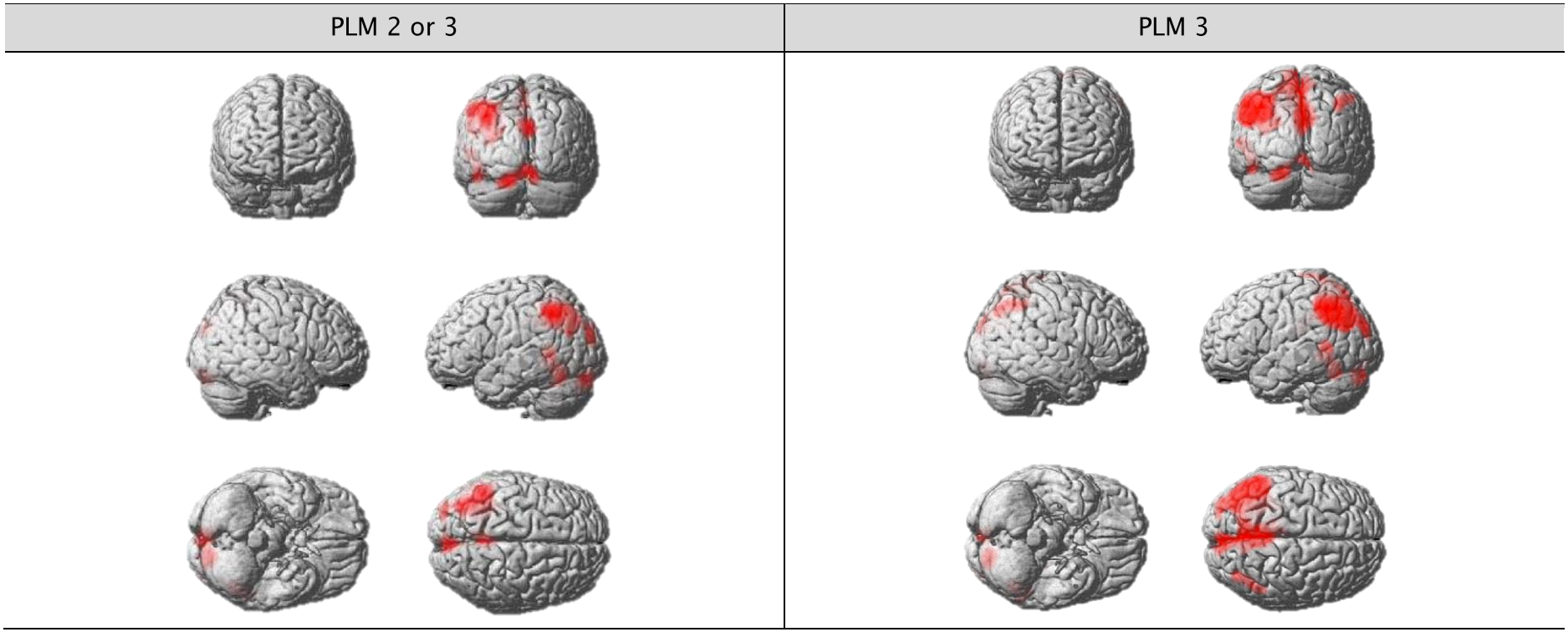


Figure 4-5 SPM comparisons of PLM high risk and low risk groups

Statistical parametric mapping (SPM) comparison showing areas of relative hypoperfusion (red) in high AD risk (PLM 2 and 3 combined, and PLM 3 only) when compared to low and very low AD risk groups (PLM 1 and 0) according to their CSF analysis results. Results are shown at $p < .001$ uncorrected and 100 voxel cluster threshold. No clusters survived at $p < .05$ FWE threshold.

4.5.2 Discriminant analysis prediction of group membership

Using discriminant analysis, regional cerebral perfusion as specified from region of interest values was highly predictive of CSF biomarker status (see Table 4-5). Importantly, these regions were not derived directly from the initial t-test results in the prior section. The angular gyrus had the highest predictive power, with the left superior occipital gyrus, posterior cingulate, left middle temporal gyrus, left superior temporal pole and the caudate and putamen also contributing to classification accuracy.

Perfusion SPECT imaging predicted any CSF abnormality with good sensitivity and specificity (87.1% specificity, 81.1% sensitivity). Those at highest risk of disease (PLM 2 and 3) were also distinguished from those at low risk (PLM 0 and 1) with reasonable accuracy (73.5% accuracy, 76.6% specificity, 66.7% sensitivity, positive predictive value 56.0%, negative predictive value 83.7%) with the left angular gyrus and right middle temporal pole providing the best discrimination between groups. Of the 68 individuals tested, 50 were correctly grouped into low or high AD risk.

Table 4-5 Discriminant analysis from PLM groups

The accuracy of perfusion ROIs most predictive of PLM group when compared to patients with normal CSF biomarkers (PLM 0; *N* = 26). L = left, R = right, PPV / NPV = positive / negative predictive values. Values in brackets represent 95% confidence intervals unless otherwise specified. * Indicates a reverse relationship (lower rCBF in normal groups).

	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Regions of interests
Any abnormality	85.7	83.8	88.5	91.2	79.3	L angular gyrus
(N = 37)	(74.61 -93.3)	(68.0 – 93.8)	(69.9 – 97.6)	(77.9 – 96.8)	(64.5 – 89.0)	L superior occipital
						R medial temporal
						Posterior cingulate
PLM 1	90.5	75	100	100	86.7	L inferior parietal
(N = 16)	(77.4 – 97.4)	(47.6 – 92.7)	(86.8 – 100.0)	(NA)	(73.6 – 93.8)	L superior occipital
						L medial temporal
						Posterior cingulate
						R middle temporal
PLM 2 & 3	84.8	81.0	88.0	85.0	84.6	L angular gyrus
(N = 21)	(71.7 – 93.8)	(58.1 – 94.6)	(69.9 – 97.6)	(65.7 – 94.4)	(70.2 – 93.4)	L superior occipital
						L caudate & putamen *
PLM 3 only	95.1	93.3	96.2	93.3	96.2	L angular gyrus
(N = 15)	(83.5 – 99.4)	(68.1 – 99.8)	(80.4 – 99.9)	(67.1 – 99.0)	(79.0 – 99.4)	L superior occipital
						L superior temporal pole *

4.5.3 Patterns in the individual patient

Further characterisation of regional abnormalities was performed to improve understanding of imaging patterns with specific diagnostic reporting significance at the single subject level. Single-subject against control group SPM map overlays for PLM groups 0, 1 and 2/3 at a threshold of $p < .001$ uncorrected are shown in Figure 4-6 and Figure 4-7.

PLM 0 showed relatively little hypoperfusion overlap, with a number of underlying perfusion patterns. This group also includes individuals with no perfusion abnormality at the chosen threshold (11/26).

PLM 1 and PLM 2/3 showed similar patterns, with the angular gyrus and precuneus regions identified as the most common regional abnormalities. The PLM 1 group showed less extensive overlap, with a left hemisphere predominant pattern and more overlapping heat spots in non-parietal regions, including the temporal pole. PLM 2/3 showed bilateral posterior cortical maximal abnormality with more generalised (extensive) overlap. Three individuals in the PLM 1 groups showed no regions of hypoperfusion at the chosen threshold (3/16). A single individual in the PLM 2/3 group showed no abnormality in the single subject maps (1/21), however repeat HMPAO SPECT imaging after a period of two years revealed significant hypoperfusion over the left angular gyrus and parietal association area at UHS standard reporting thresholds ($p < .001$ uncorrected).

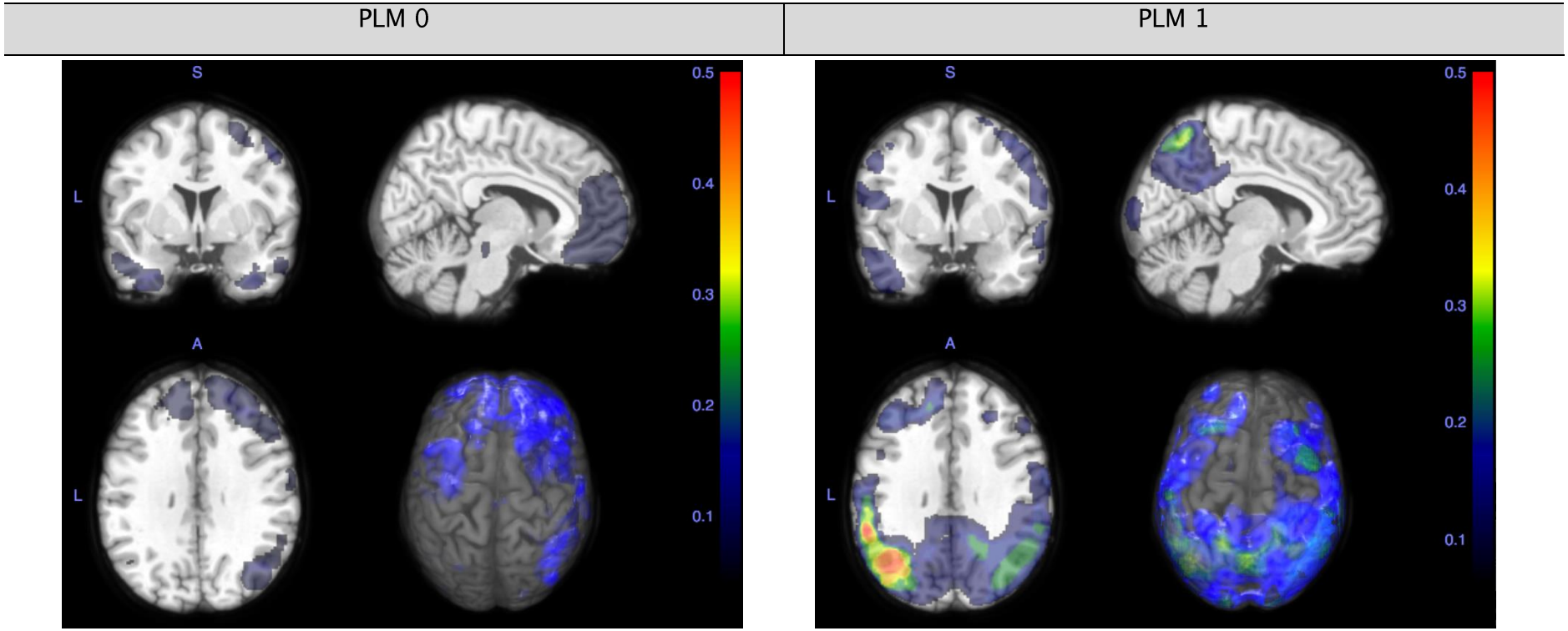


Figure 4-6 Overlay maps of single-subject SPM analyses for PLM groups 0 and 1

Single subject against control group SPM t-tests ($p < .001$ uncorrected with 100 voxel cluster threshold) were completed and individual patient hypoperfusion patterns binarized and overlaid for each PLM group. Scale shown is the proportion of patients in the group that showed hypoperfusion in each region. All regions with two or more patients with perfusion abnormality in the same location are shown.

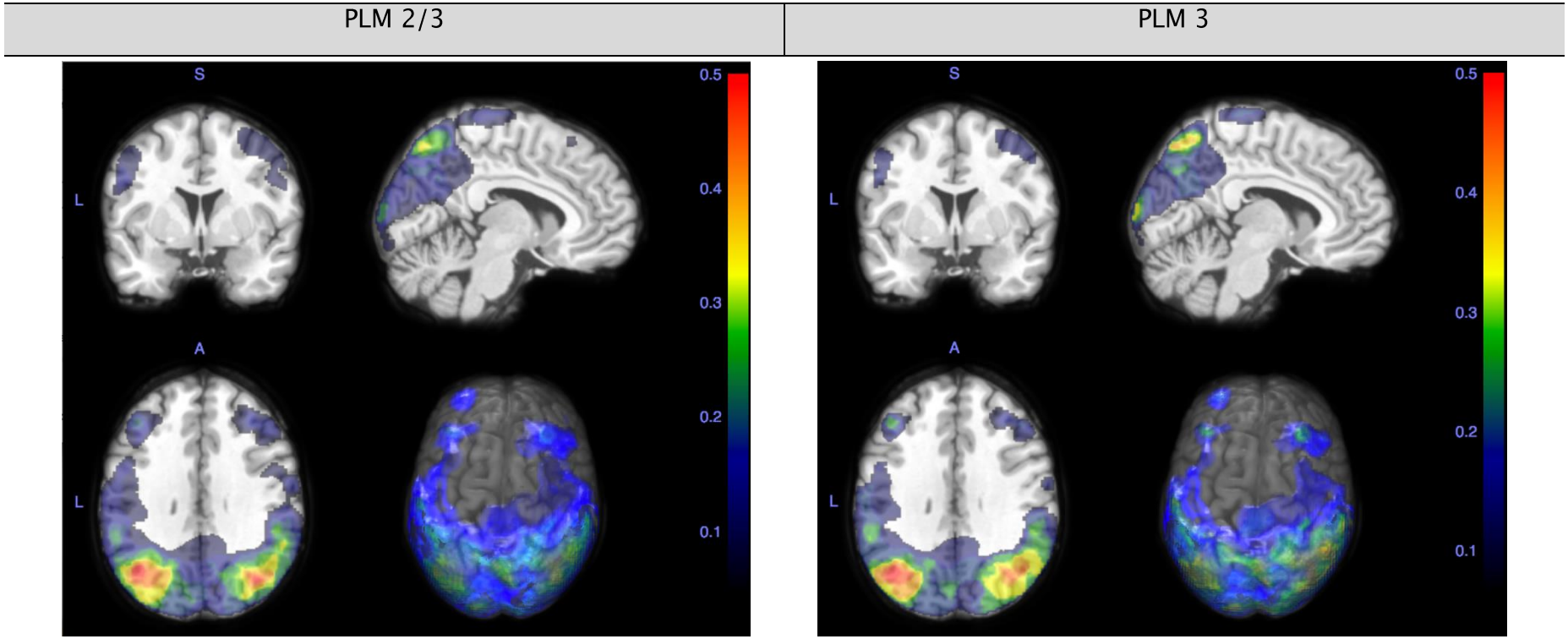


Figure 4-7 Overlay maps of single-subject SPM analyses for PLM groups 2/3 and 3

Single subject against control group SPM t-tests ($p < .001$ uncorrected with 100 voxel cluster threshold) were completed and individual patient hypoperfusion patterns binarized and overlaid for each PLM group. Scale shown is the proportion of patients in the group that showed hypoperfusion in each region. All regions with two or more patients with perfusion abnormality in the same location are shown.

4.6 Discussion

CSF biomarkers are effective at predicting AD pathology, however their use is limited by clinical availability, need for careful handling and the invasive nature of their collection. The ability to use functional imaging to accurately and reliably identify AD pathology, as defined by CSF biomarker status has significant clinical utility. In this study, we show that regional perfusion deficit on HMPAO SPECT can predict AD biomarker status on an individual subject level, identifying regions most strongly associated with AD-type biomarkers.

Regional abnormality of the angular gyrus and precuneus were the most salient indicators for AD-type CSF biomarkers and correlated well with a combined CSF AD risk scale (PLM scale) both on group analyses and visualisation of individual patient perfusion patterns. Importantly, these findings were demonstrated in a mixed clinical sample of patients with cognitive complaints defined by diagnostic uncertainty. The presence of angular gyrus and precuneal hypoperfusion in AD is consistent with current knowledge of AD imaging patterns (see Chapter 1).

When patients were grouped by biomarker status, all PLM groups showed significant deficits when compared to controls ($p < .001$ FWE corrected), but a dose-response relationship could be seen with increasing number of AD-consistent biomarkers more highly correlated with 'typical' AD perfusion pattern imaging. The angular gyrus was most predictive in differentiating between patients with AD-type and non-AD type pathology. High accuracy (84.8%) was shown when differentiating between those at very low risk of AD (PLM 0) and those at highest risk (PLM 2/3) using discriminant analysis, highlighting the potential of a combination of regions to predict AD type CSF profiles. Although lower accuracy was obtained when predicting those at highest risk of AD (PLM 2/3) from those at lower risk (PLM 0 and PLM 1), the majority of individuals were correctly classified (50/68). This lower accuracy is likely due to the PLM 1 group containing both those with and without AD, therefore reducing the discrimination power of AD type regional abnormalities.

Overlay maps of individuals at highest risk of AD (PLM 2/3) showed bilateral perfusion deficits in the angular gyrus, with less frequent overlap of regional abnormality in the frontal and temporal regions. The extension of

hypoperfusion into frontal and temporal regions highlight the variability in individual cases. Superior frontal imaging abnormality has been shown to be present in AD individuals, often presenting in combination with parietal deficits (66,245). In addition, the medial temporal lobe is a well described locus for imaging abnormality in AD, although the ability of HMPAO SPECT to detect abnormality in the medial temporal lobe is debated (see Chapter 1). Of note, the presence of specific regional perfusion deficits, particularly the angular gyrus, appeared to be more important than the extent of the hypoperfusion in predicting CSF status. A similar pattern of overlap was seen in the PLM 1 group, with a focal region of overlap in the left angular gyrus and small overlap loci in the superior frontal lobe. The similarity of patterns seen in the PLM 2/3 and PLM 1 group is likely due to a proportion of individuals in the PLM 1 group having AD pathophysiology, in addition to individuals with non-AD dementias, potentially including those with a suspected non-AD pathologic process (SNAP) and mixed type pathologies (246,247).

Single subject SPM overlay maps can provide clinical and diagnostic information at an individual level, but have not previously been validated in HMPAO SPECT in relation to underlying pathology. This study shows that involvement of the angular gyrus and precuneus for an individual subject is significantly related to abnormal CSF biomarkers, and in turn, risk of underlying Alzheimer's disease pathology. This has implication for clinical diagnostic reporting of single subject images based on voxel-based analysis, facilitating improved confidence in an AD diagnosis when these regions are abnormal in the individual. Further definition of common patterns and their associated biomarker status in a mixed clinical sample where diagnostic doubt exists may provide added utility for clinical reporters in cases where HMPAO imaging patterns are ambiguous. The results from this study were not compared to interpretations from non-quantitative (visual) analysis, so this study cannot demonstrate improved accuracy relative to visual reporting methods. A head-to-head comparison of these reporting methods has however previously been completed (36).

A recent study by Andriuta et al (2018) comparing HMPAO SPECT imaging and CSF AD biomarkers found that the ratio of the inferior parietal cortex to dorsolateral frontal cortex provided moderate prediction accuracy for CSF biomarkers (receiver operator curve area under the curve 0.648 – 0.671) when

cut-offs were optimised using the Youden index (243). Our results agree that the inferior parietal cortex can provide discrimination power for AD individuals with abnormal CSF biomarkers, however they also suggest that the use of the posterior to frontal ratio may not provide optimal classification. As shown in both the group and individual analyses in our study, frontal hypoperfusion can present in AD cases, affecting positive prediction when this region is included in regression or discriminant analysis. This is highlighted by the poor positive prediction values found in the Andriuta et al study (33% to 46%). The use of a range of regions across the brain as shown in this study could provide increased classification accuracy, although prediction accuracy would need to be tested on an independent sample to ensure validity.

The PLM scale was used in this study due to its simplicity and high diagnostic accuracy compared to CSF biomarkers used alone (241,248), although other frameworks to define CSF abnormality could have been used. A recent paper completed by the authors of the PLM scale found that the inclusion of Aβ42/40 ratio in the scale (PLM_R scale) may provide further clarification for individuals with discordant (PLM 1 and PLM 2 cases) CSF results (249). Although the revised scale reduced the proportion of discordant results, they did not find a significant increase in prediction accuracy over the original PLM scale. In this study, we did not have access to CSF Aβ42/40 ratios. The A/T/N framework proposed by Jack and colleagues (2016) which divides patients based on the binary categories Aβ42 (A; CSF or amyloid PET), tau (T; CSF P-tau or tau PET), and neurodegeneration (N; CSF T-tau, MRI atrophy, HMPAO SPECT or FDG PET) could have also been used in this study (246). Due to the increased number of potential categories in the A/T/N framework compared to the PLM scale (eight possible outcomes compared to four) and the relatively modest sample size in this study, the PLM scale was chosen as a more appropriate method of classification in this case. This study was not however completed to explore the validity of the PLM or other classification framework, but to validate AD HMPAO SPECT patterns.

This study does have limitations. It was a retrospective sample, and there may be bias in the selection of individuals receiving both HMPAO SPECT and CSF biomarker assessments. We have no clinical measures of severity for confirmation of the stage of the disease. Individuals in the PLM 2/3 group may be more severely affected, thus influencing the extent of group abnormality on

SPM maps. Countering this was the observation in several patients that extent of perfusion deficit on individual SPM perfusion maps did not appear to be related to the number of abnormal biomarkers. Regional specificity was more important than extent of disease. As mentioned in Chapter 1, SPM single subject against control group analysis is also inherently sensitive to database variation and can produce false positive results when applied to structural imaging (41,42). A larger control group or the use of non-parametric methods may minimise the risk of false positives. Without histopathological verification of diagnosis, long term follow up validation is needed for cases where imaging and CSF results conflict. Longitudinal outcomes of regional perfusion imaging deficit is addressed in more detail in Chapter 1 and Chapter 4. Misclassification may also occur where patient pathology is near cut-off thresholds for either CSF abnormality or SPECT hypoperfusion.

In conclusion, regional abnormality on perfusion SPECT imaging is associated with CSF AD biomarker levels and perfusion patterns differ for and can be used to discriminate between those with low or high AD risk. Regional abnormality of the inferior and medial parietal lobe (angular gyrus and precuneus) is highly predictive for AD type CSF biomarkers and can be used in combination with other regions to accurately identify CSF abnormality. This information can be used to increase confidence in those reporting individual perfusion deficit maps that specific regional abnormalities correlate well with underlying Alzheimer's disease pathology.

Chapter 5: Longitudinal validation of rCBF imaging patterns

5.1 Introduction

Accurate prognosis for patients with cognitive complaints is clinically important, aiding signposting of individuals to appropriate care resources in addition to allowing the patient and family members to prepare and plan for the future. Survival time for individuals with Alzheimer's disease has been shown to be nearly half that of the general population in certain age groups, with patients that show the greatest decline in the first year of follow-up having a significantly increased risk for death (250). The ability to identify individuals who are at highest risk of poor survival could be utilised in clinical practice to ensure provisions are in place to support both the patient and their family.

As shown in the previous Chapters, perfusion SPECT imaging patterns have good diagnostic accuracy when validated with independent biomarkers, with the location of cerebral perfusion deficits allowing identification of dementia subtype. Although the relationship between perfusion abnormalities and dementia pathology is known, the accuracy of perfusion SPECT abnormality to identify individuals who are likely to decline in a clinical sample is less clear.

Numerous studies have investigated the imaging patterns of patients who decline to dementia or stay stable over time, with results varying. Jagust et al (1998) found that right parietal perfusion predicted overall survival in a group of 50 patients with Alzheimer's disease, with those with the lowest rCBF in the right parietal region having a significantly shorter survival than other patients (174). Deficits in the temporoparietal, posterior cingulate and medial temporal regions have been found to consistently show good prediction accuracy in distinguishing between individuals with mild cognitive impairment that do, or do not, progress to Alzheimer's disease (progressive MCI and stable MCI), with prediction accuracy (ROC AUC) ranging from 0.75 to 0.82 (78,79,99,100,161,165–167). Frontal and other temporal regions have however also been shown to predict progression (96,101), in addition to some studies finding no difference in rCBF between decliners and non-decliners

(92,170,251). Correlations between rCBF and decline on cognitive scores such as MMSE and disease severity scores such as CDR have also been performed, showing a relationship between reduced perfusion on baseline SPECT and future decline on test scores (168,171,175,176).

The above studies all aim to aid identification of patients at a preclinical stage of dementia who are likely to progress, however they focus on samples that progress to or decline from AD exclusively, omitting individuals who progress to non-AD dementia subtypes such as frontotemporal dementia or dementia with Lewy bodies. Additionally, although these studies often use clinical cohorts, they are rarely naturalistic, with stringent classification of dementia subtypes often implemented before analysis is completed. There has been little exploration of the utility of SPECT perfusion patterns to identify future decline in clinically useful, unselected samples using quantitative analysis techniques.

In this chapter, we completed an exploratory analysis which aimed to validate HMPAO SPECT imaging for dementia detection using longitudinal follow up and investigate the relationship between specific perfusion patterns and decline, regardless of dementia diagnosis, for those with cognitive complaints using images obtained as part of normal clinical care.

5.2 Hypothesis

1. Abnormality on HMPAO SPECT imaging corresponds strongly to longitudinal decline.
2. Decline differs depending on the pattern of abnormality seen on HMPAO SPECT imaging.

5.3 Contributions and Collaborations

The study was conceived and designed by the author and Dr C. Kipps. Participant recruitment was via the longitudinal HMPAO SPECT study Brain imaging in dementia study (BraIID; see Chapter 2 for further details). Participant consent for the BraIID study was carried out by the author and Ms S. Varkonyi, and ethical approval for the study was obtained by the author. Initial

image processing for HMPAO SPECT imaging was performed by Dr L. Tossici-Bolt, Dr S. Michopoulou and Mr E. Varzarkis. All subsequent data processing and statistical analysis was performed by the author. Interpretation of the data and preparation of tables and figures was carried out by the author.

5.4 Methods

5.4.1 Data collection

5.4.1.1 Sample

Subjects were recruited through the NIHR portfolio BrainID study. As described in the methods and BrainID protocol (see Appendices), the BrainID study is an observational study that aims to maximise the use of information collected as part of normal clinical care to provide clinically valid information on perfusion SPECT imaging to aid clinician diagnosis and prognosis. All patients who had HMPAO SPECT imaging completed at UHS after November 2015 as part of their clinical care due to cognitive complaints were offered the opportunity to take part in the study. The vast majority of patients were recruited on the day of their scan, although the protocol allowed consent up to three months before or after imaging was completed. To ensure participants covered the whole spectrum of dementia and cognitive complaints, those who lacked capacity to consent to the study were recruited using a consultee (for example a spouse or close friend of the patient) if appropriate. If a patient did not have capacity and no consultee who knew the patient in a personal sense was present, the patient was not consented to the study.

Companions of patients present at the time of consent were also asked to participate in the study to provide longitudinal follow up information on the patient throughout the study.

5.4.1.2 Clinical data collection

At the time of study consent, patient participants were asked to complete a short form giving basic demographic information, past medical history and medications taken. If the patient was assessed to lack capacity to consent, their companion was asked to complete this initial form. To minimise burden

on the participant, all subsequent follow up was completed through the companion.

Companion participants were asked to provide information on the patient at time of consent and at 6 monthly follow up intervals through the completion of the following questionnaires by telephone or post:

- Clinical Dementia Rating sum of boxes (CDR-SoB) symptom inventory
- Cambridge Behavioural Inventory (CBI) behavioural assessment
- Zarit caregiver burden questionnaire (Zarit)
- Functional Activities Questionnaire (FAQ)

Outcome data on progressive change from the follow up questionnaires was obtained in addition to patient diagnosis and date of death (if relevant).

5.4.1.3 Image acquisition and processing

Consent to the BraIID study allowed collection of HMPAO SPECT images for all participants. Perfusion SPECT images were acquired on a GE Infinia 3/8 HK4 dual head gamma camera, with parameters of acquisition and reconstruction outlined in Chapter 2 (Methods). Images were spatially normalised, smoothed and cerebellar normalised using statistical parametric mapping software (SPM8) (38). Correction for age was applied. Regions of interest (ROIs) were created for the frontal, parietal, temporal and occipital lobes using the automatic anatomical labelling (aal) atlas and WFU-pickatlas software (206-208). Measures of perfusion were calculated from the HMPAO SPECT images for the ROIs using SPM8 and Marsbar software (209).

5.4.2 Statistical analysis

We hypothesised that regional and global perfusion on HMPAO SPECT corresponds to longitudinal decline. Decline was defined as an increase on the CDR-SoB scale greater than or equal to 2 points per year. This outcome measure was determined through both clinical expertise (Dr Christopher M. Kipps) and from the literature, where intermediate AD decliners were noted to gain around 2 points per year on the CDR-SoB (252). CDR sum of boxes score was used instead of CDR global score as it affords larger distinction in severity between individuals, providing a more sensitive scale for severity change (198).

Patients were classified into abnormal and normal groups when compared to a normal control group (see Methods Chapter for control details) for both global and ROI signature pattern analysis using measures of perfusion. For global abnormality groups, patients were classified as abnormal if there was a two standard deviation or larger reduction of perfusion when compared to controls in one or more of the pre-defined ROIs. Individuals classified as abnormal were further divided into pattern groups defined based on the region found to have the largest z-score difference from controls. This was defined on the basis that the region with the largest perfusion deficit was most likely to be the location where the perfusion abnormality originated.

Chi-square tests were completed to compare proportions of individuals in pattern groups with the CDR-SoB outcome. Cerebral perfusion of decliners was compared to non-decliners in SPM voxel based analysis using both a $p < .001$ uncorrected and $p < .05$ Family wise error multiple comparison corrected with 100 voxel cluster threshold.

Survival analysis was completed to determine difference in time to event between global abnormal and normal and regional pattern groups. Kaplan-Meier curves were created to assess differences in decline rates between groups. Survival time was measured from date of study consent. ROI z-score against control group means were produced to further explore individuals who were classified as normal on imaging however reached the decline outcome.

5.5 Results

5.5.1 Sample demographics

A total of 78 participants were recruited between November 2015 and July 2016, and were followed up until August 2017. Sixty-six of these participants had at least two completed CDR questionnaires and available perfusion SPECT scans for analysis, with 4 individuals withdrawing and 5 lost to follow up. Three individuals died during follow up. Demographic information on the sample is presented in Table 5-1. The sample had a larger proportion of male patients than female (67%), with the majority of caregivers being female (70%) and a spouse or partner of the participant (82%). All caregivers knew the

participant in a personal sense. The mean age of participants was 68.7 years old (S.D. 8.66), and most were living with their spouse or partner at the time of imaging (88%) although some were living alone (7.5%, 4.5% living status unknown). Patients were on average rated 'mild' on the CDR global score (median = 0.5, range 0 – 2), and had a mean score of 10.85 (S.D. 8.66) on the FAQ rating scale (out of a total of 30). Most individuals were right-handed (91%) and participants were on average well educated (Mean = 12.5 years of education, S.D. = 3.83, *N* = 54). Caregivers had on average mild to moderate burden as rated by the ZBI (mean = 25.5, S.D. = 18.1).

For individuals followed throughout the study, 10 participants had 6 months of follow up, 34 participants had 12 months and 22 participants had 18 months or more. Twenty-two of the participants with follow up information reached the primary endpoint of a decline of two points or more (33%). Among these participants, twelve reached the primary endpoint within 6 months, eight within a year and three after 18 months of follow up.

Table 5-1 Sample demographics

Demographics and severity and functional scores of the sample when grouped into those with abnormal (defined as >=2 S.D. away from control group mean) or normal perfusion in any region. T-tests, Mann-Whitney U and Chi-square analysis was completed where appropriate to compare groups. No significant differences were found between abnormal or normal groups in any variable displayed ($p > .05$). * CDR and CDR-SoB was unavailable at baseline for three participants. ** FAQ score and ZBI score was unavailable at baseline for six participants.

	Global abnormal	Global normal
N	38	28
Patients M/F	26/12	18/10
Companions M/F	12/26	8/20
Patient handedness R/L (Unknown)	36/1 (1)	24/2 (2)
Patient age in years (mean ± S.D.)	69.53 ± 7.73	67.61 ± 9.84
Follow-up in days (mean ± S.D.)	413.47 ± 143.09	434.96 ± 129.41
Patient global CDR (median with range)*	1 (0 – 2)	0.5 (0 – 1)
Patient CDR-SoB (mean ± S.D.)*	4.92 ± 2.86	3.92 ± 2.72
Patient FAQ score (mean ± S.D.)**	12.28 ± 7.62	9.15 ± 7.10
Caregiver Zarit Burden score (mean ± S.D.)**	28.27 ± 17.45	22.15 ± 18.58

5.5.2 Imaging patterns and events

Of the 66 patients involved in the analysis, 38 (58%) were found to have a perfusion abnormality greater than two standard deviations away from controls in one or more of the ROIs, with 28 classified as normal (42%). Of those with abnormal perfusion, 14 were defined as having peak abnormality in the frontal region, 9 as parietal, 4 as temporal and 11 as occipital.

A chi-square test of independence was performed to examine the relationship between perfusion SPECT global abnormal or normal rating and the clinical outcome (2 points decline on the CDR-SoB). The relationship between these variables was significant (χ^2 (1, N = 66) = 5.24, p < .05). Imaging prediction of decline showed a specificity of 52.3%, sensitivity of 81.0%, positive predictive value of 44.7%, negative predictive value of 85.2% and overall accuracy of 61.5%. Those with abnormal perfusion SPECT were significantly more likely to reach the endpoint than those with normal imaging, with 45% of individuals with abnormal imaging reaching the endpoint compared to 18% of normal. Mean CDR sum of boxes score at each follow up visit for the SPECT global abnormal and normal groups are shown in Figure 5-1.

When clinical outcome proportions were compared between regional patterns there was no significant difference in the number of individuals reaching the endpoint between pattern groups (χ^2 (4, N = 66) = 6.12, p > .05).

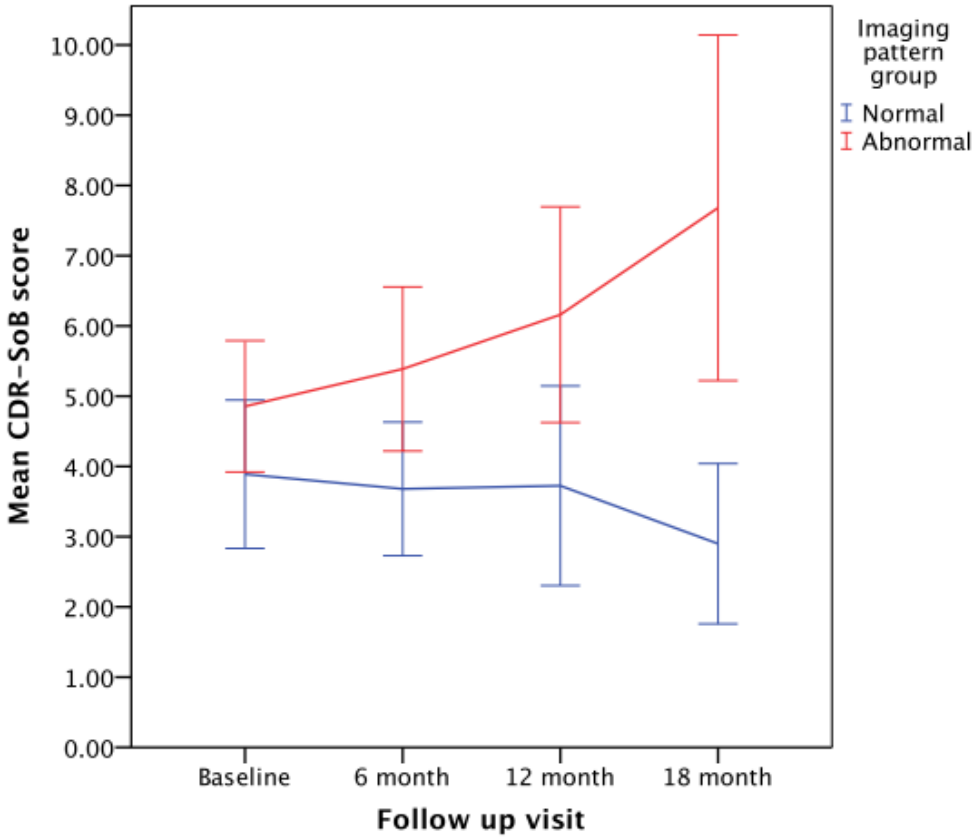


Figure 5-1 CDR-SoB scores for normal and abnormal perfusion SPECT groups

Mean CDR-SoB scores for normal and abnormal perfusion SPECT groups at each follow up point are shown. Images were classified as abnormal or normal based on any perfusion deficit in frontal, parietal, temporal or occipital regions 2 standard deviations or more away from the control group mean. Error bars shown indicate 95% confidence intervals.

5.5.3 SPM analysis of decliners against non-decliners

SPM analysis of individuals with cognitive complaints that reached the outcome compared to those that did not reach the outcome are shown in Figure 5-2. Individuals with cognitive complaints showed significantly reduced perfusion bilaterally in parietal and occipital areas, including the angular gyrus and precuneus regions at $p < .001$ uncorrected threshold. A small area in the superior medial frontal lobe around the left supplementary motor area was also found to be significantly different between groups at the cluster level. At the more stringent threshold with multiple comparison correction ($p < .05$ FWE), a single significant cluster remained in the left precuneus region. No significant regions of reduced perfusion for non-decliners when compared to decliners were found.

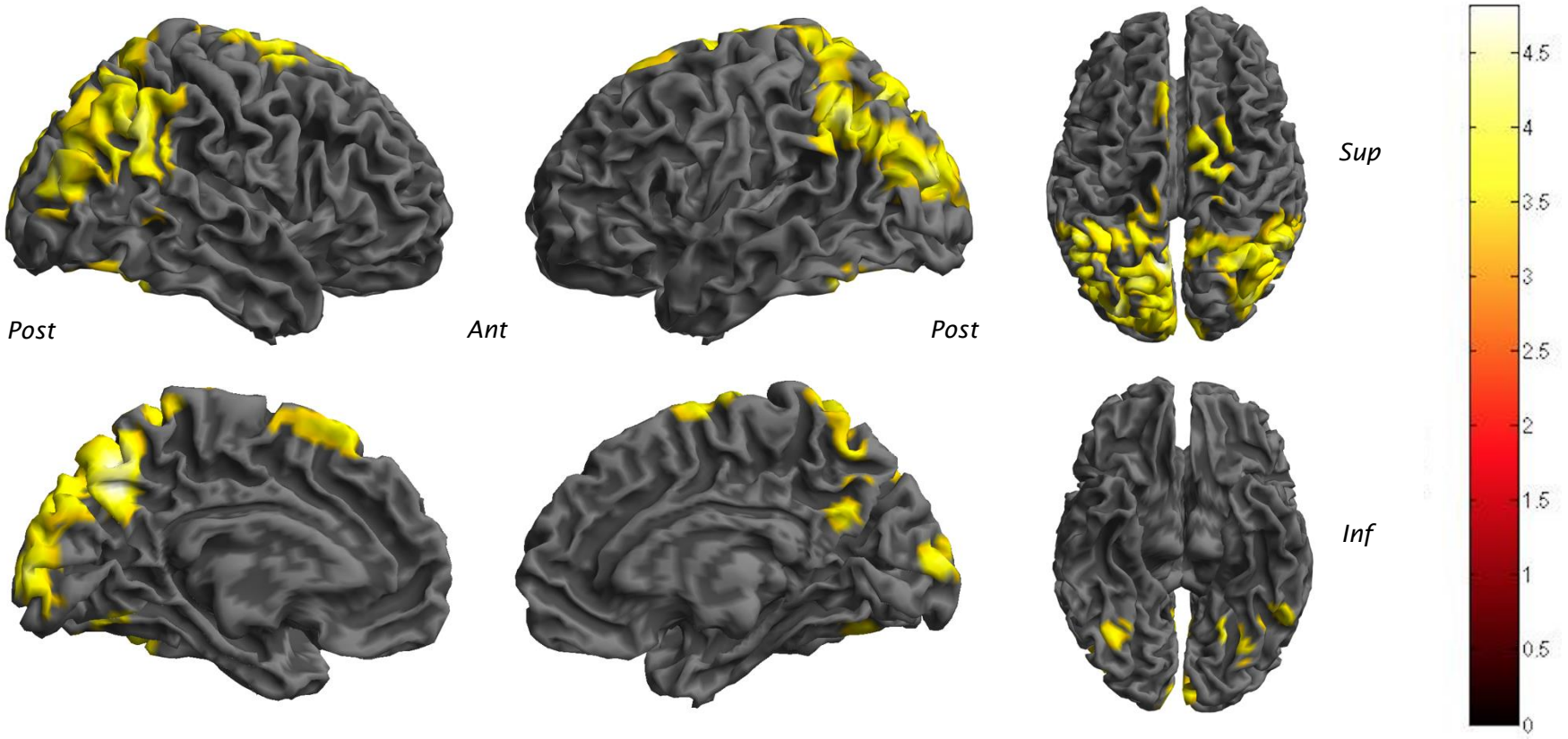


Figure 5-2 SPM comparisons of decliners and non-decliners

Decliners were classified as individuals who had a decline on the CDR-SoB of two or more points in a year. Results shown are at a $p < .001$ uncorrected threshold with 100 voxel cluster threshold with a heat scale (t-scores) indicating areas where decliners had reduced perfusion compared to non-decliners. Post = posterior, Ant = anterior, Sup = superior, Inf = inferior.

5.5.4 Survival distributions

5.5.4.1 Any and no abnormality group time to event

When survival distributions were investigated using survival curve (Kaplan-Meier) analysis and the Log Rank test, there was a statistically significant difference between distributions for those with any abnormality on their perfusion scan compared to those without abnormality (χ^2 (1, N = 66) = 8.12, p < .01) (Figure 5-3). Those with rCBF abnormality in any ROI had a one year survival probability of 63%, compared to 88% survival probability for those with normal perfusion in all regions.

5.5.4.2 Pattern group time to event

Investigation of pattern decline rates using the Log Rank test showed a statistically significant difference between the distributions of pattern groups (χ^2 (4, N = 66) = 10.60, p < .05). Kaplan-Meier curves for pattern groups can be seen in Figure 5-4, with survival probabilities and the number of events in the sample for each group shown in Table 5-2.

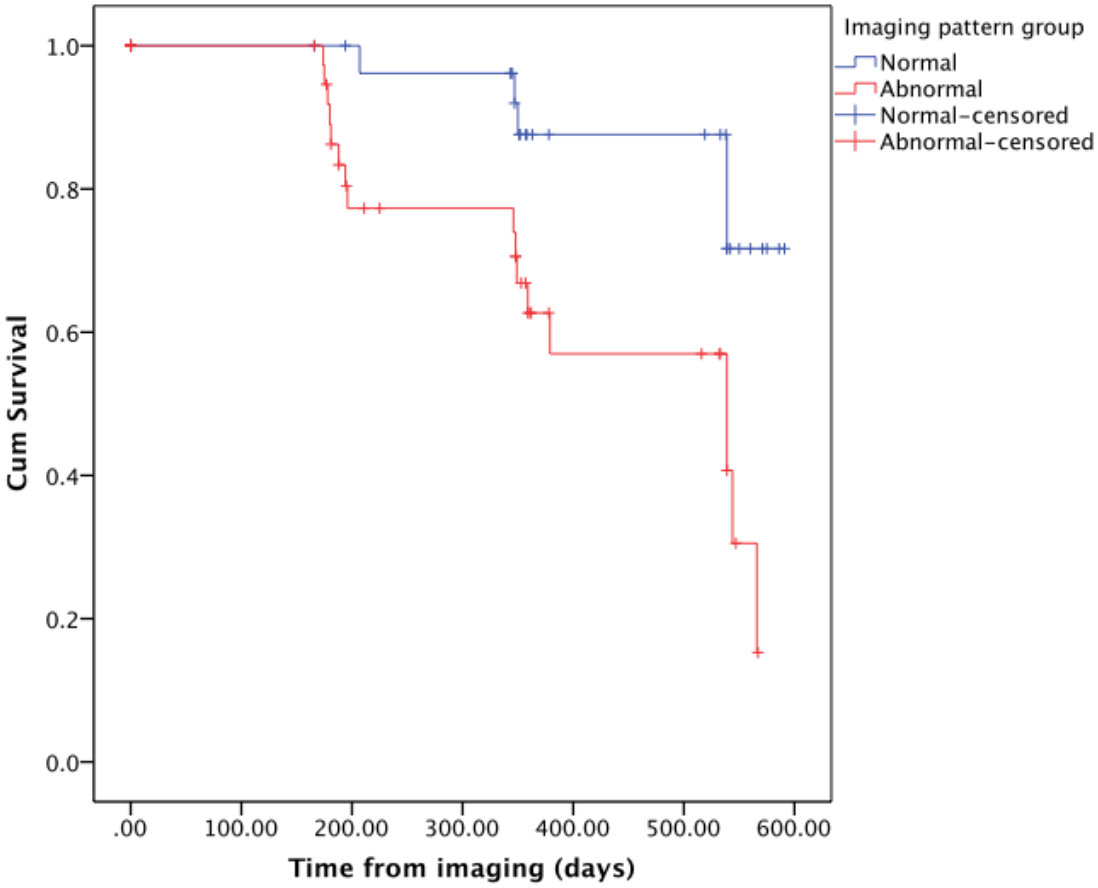


Figure 5-3 Kaplan-Meier curve comparing normal and abnormal perfusion SPECT group decline rates

Individuals were classified as normal or abnormal based on any perfusion deficit in frontal, parietal, temporal or occipital regions 2 standard deviations or more away from the control group mean. Outcome was defined as a 2 point per year decline on the CDR-SoB scale.

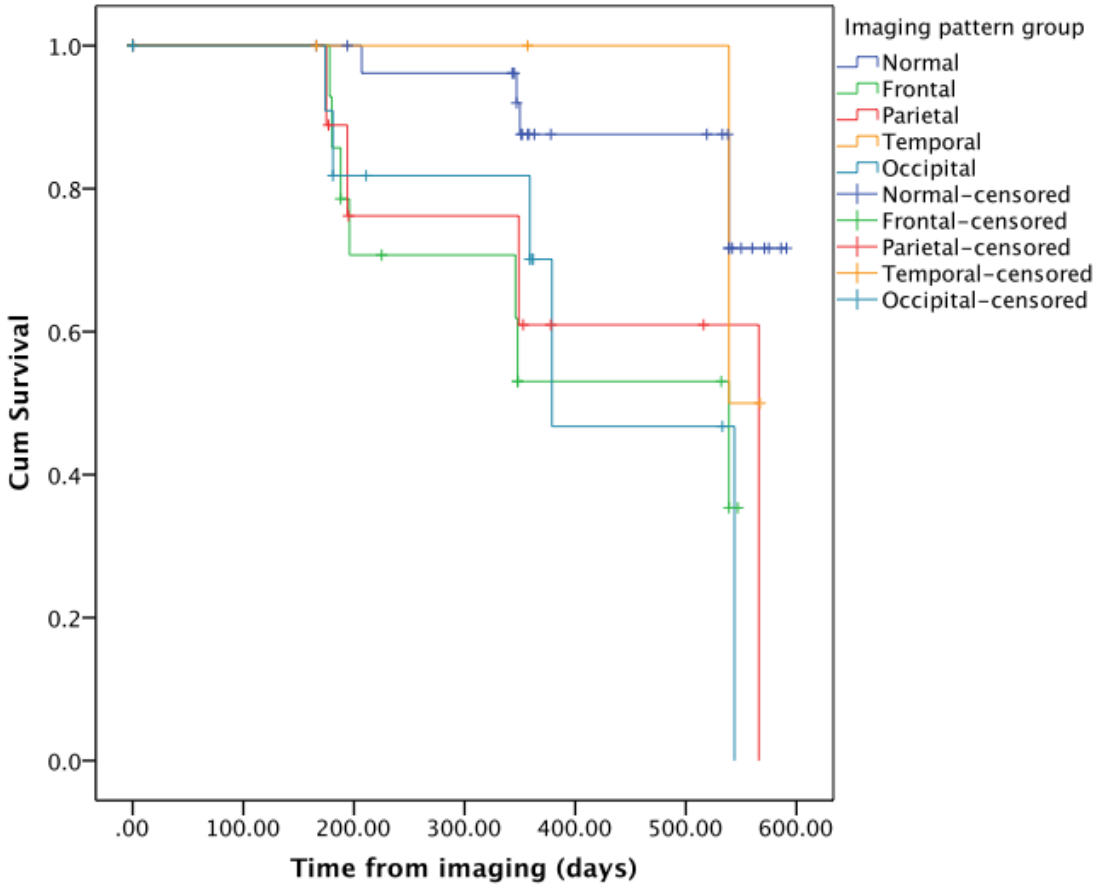


Figure 5-4 Kaplan-Meier curves for perfusion SPECT imaging regional patterns

Individuals were classified into groups based on z-scores away from controls. Individuals with z-scores smaller than 2 standard deviations from controls in all regions were classified as normal. Abnormal individuals were divided into frontal, parietal, temporal and occipital originating perfusion deficit based on the region with the largest z-score from controls (i.e. largest perfusion deficit).

Table 5-2 Predicted survival probabilities for pattern groups

Patterns were defined based on the region found to have the largest z-score difference from controls. Normal was defined as less than 2 standard deviations away from control mean in all regions of interest. Survival represents those who did not reach the CDR outcome.

	Pattern groups				
	Frontal	Occipital	Temporal	Parietal	Normal
N	14	11	4	9	28
Events, N (% of total in group)	7 (50%)	5 (46%)	1 (25%)	4 (44%)	5 (18%)
6-month survival	86%	82%	100%	89%	100%
12-month survival	71%	70%	100%	61%	88%
18-month survival	36%	47%	50%	61%	72%

5.5.5 Individuals with normal perfusion who reached the outcome

Five individuals classified with normal brain perfusion met the outcome in our sample. Exploring the z-scores for these participants compared to controls shows that three of the five individuals had perfusion deficits that were only marginally below the normal/abnormal threshold of 2 S.D. away from controls (Table 5-3).

Reducing the imaging cut-point to 1.5 S.D. away from controls reduced the overall number of individuals in the normal group to 20 (from 28), with two of these reaching the decline endpoint (10%). The reduced cut point increased sensitivity for prediction of decline to 95.2% and negative predictive value to 94.7%, however decreased specificity to 40.9% with similar positive predictive value (43.5%). Overall accuracy decreased to 57.6%. The difference between proportions of patients who reached the outcome for normal and abnormal groups using the lower cut-off was very similar to the higher 2 S.D. cut-off (χ^2 (1, N = 66) = 8.71, p < .01).

Table 5-3 Z-scores for patients with normal imaging who reached the study outcome

Z-scores for each ROI are shown. Imaging was classified using a two standard deviation away from control mean cut-off. Five individuals who were classified with normal imaging reached the decline outcome of at least 2 points decline per year on the clinical dementia rating sum of boxes scale.

ID	Frontal	Occipital	Temporal	Parietal
106	-1.16	-1.34	-0.34	-1.91
112	0.33	-1.24	-1.15	-1.10
118	-1.77	-1.08	-1.97	-1.74
137	-1.95	-1.90	-1.78	-1.82
141	-0.32	-0.51	-0.92	0.30

5.6 Discussion

The ability to offer an accurate prognosis for patients with cognitive complaints is valuable for patients, carers and clinicians. In this study, we show that perfusion SPECT abnormality strongly corresponds to cognitive decline in an unselected clinical sample. We found that individuals with any abnormal perfusion showed a significantly faster time to event and worse survival probability than patients with normal perfusion, clearly identifying those with degenerative conditions. Individuals with normal imaging rarely reached the decline outcome. Additionally, preliminary analysis on differences in survival between perfusion patterns suggests that perfusion SPECT may be able to provide further prognostic information for the individual in a clinical environment.

Kaplan-Meier survival curves showed that patients with normal perfusion had a significantly increased survival probability and decreased likelihood of reaching the decline outcome compared to those with an abnormal rCBF in any region. Those with normal rCBF in all regions had a one year survival probability of 88%, compared to 63% survival probability for those with abnormal perfusion. These survival differences were found despite no significant difference between groups in scores for CDR, FAQ or Zarit burden questionnaires at baseline. A prior study by Hansson et al (2009) found similar results when investigating cerebral blood flow using a ^{133}Xe tracer SPECT method and following individual decline over 4 to 6 years. When pathological imaging was defined by reduced rCBF in parietal regions only, those with abnormal imaging had a hazard ratio of 3.92, with decreased rCBF being a significant risk for future development of AD. Although Hansson et al had a long follow up period, patients with MCI who progressed to non-AD dementias were excluded from the analysis. In addition, Hansson et al (2009) defined abnormal cerebral perfusion by parietal regions only, which could misclassify patients with atypical AD regional perfusion deficits. A study by Huang et al (2003) did explore perfusion patterns for MCI patients who declined to any dementia subtype, including a small number patients who declined to FTD, vascular dementia and non-specific dementia in addition to AD (73). They found that the left parietal region could predict decliners from non-decliners with 75% accuracy, although they did not explore decline rate or survival.

The differences in survival probabilities for reaching the CDR outcome between patients with abnormal and normal imaging in the present study show that perfusion SPECT can differentiate between individuals that have a high probability of decline in functional ability from those who are likely to stay stable in a mixed clinical sample where prior diagnosis is unknown. We also note that level of prior investigation for patients referred for diagnostic imaging varies, with diagnostic uncertainty present in the sample despite cognitive and other testing (for example CT and MRI) prior to HMPAO imaging. HMPAO SPECT offers the ability to make good prognostic judgements, irrespective of the level of enrichment of the cohort prior to assessment.

We found HMPAO SPECT to have reasonable accuracy for detection of decline (61.5%), but specificity and PPV was poor. This is likely due to limited follow-up duration rather than a genuine lack of specificity with HMPAO SPECT imaging, as shown by high specificity obtained in previous Chapters for DLB and AD detection when compared against gold standard antemortem biomarkers. Extended follow up would further validate the study results. Although we found good sensitivity for detection of decline, five individuals in our sample classified as having normal imaging met our decline outcome. When regional perfusion was investigated further, it was found that three of the five patients had z-scores in at least one ROI that was very close to the cut-off point of 2 S.D. away from control mean (< -1.9 S.D. compared to controls). Reducing the cut-off point to 1.5 S.D. away from controls provided an increase in sensitivity and negative predictive value due to fewer individuals classified as normal reaching the decline endpoint, however reduced specificity for prediction of decline. Optimisation of the HMPAO SPECT cut-points used could provide added accuracy in differentiating between decliners and non-decliners. Due to the heterogeneity of perfusion SPECT imaging (as described in Chapter 1), cut-offs are not always applicable across sites, with this shown in the large cut-off variability seen in the literature. Knowledge of the sensitivity, specificity, positive and negative predictive values is essential for clinicians to prevent misinterpretation of biomarker prediction results.

When patients with abnormal imaging were grouped based on the region with the highest z-score from controls, we found significantly different survival probabilities for reaching the CDR outcome between frontal, parietal, occipital, temporal and normal groups. Frontal, parietal and occipital groups showed

similar survival probabilities in Kaplan Meier curves and likelihood of reaching outcome, showing significantly reduced survival in comparison to normal patients. Interestingly, the temporal group showed no significant difference in survival compared to the normal group and showed a longer 1 year predicted survival probability than the other abnormal regional groups. Although the temporal group showed better survival than other abnormal groups, results from the patterns analysis presented is exploratory and limited by small group sizes. No prior studies have investigated differences between perfusion SPECT pattern decline rates or survival, although studies investigating perfusion differences between decliners and non-decliners have suggested a correlation between temporal rCBF and decline on cognitive scores (79,100,176,253), particularly of the medial temporal regions (78,99,171).

The increased time to event seen for individuals with temporal patterns when compared to other abnormal patterns in our sample could be due to the method of pattern grouping. Defining patterns by the region with the largest perfusion deficit from controls may not be optimal, with the potential for decline in perfusion varying across the cerebrum masking the origin of pathology. Grouping patients by the largest z-value region may also be poorly sensitive to deficits in the medial temporal lobe due to the low spatial resolution of the SPECT imaging technique, and a specific medial temporal lobe ROI may provide better distinction between AD and FTD individuals with temporal deficits. Finally, the ROI method used in this study may also combine patterns that are visually different and misclassify individuals with mixed regional pathology (such as temporoparietal or parieto-occipital), those with patchy deficits across the cerebrum indicative of vascular pathology or individuals with unusual patterns of perfusion. The use of a visual or automated pattern grouping analysis, for example using principal component analysis or machine learning techniques such as support vector machines, may provide a more accurate grouping that could show further utility in clinical practice.

Direct comparison of perfusion SPECT imaging between decliners and non-decliners using SPM voxel based analysis found a significantly lower perfusion for decliners in parietal and occipital regions, with a cluster in the precuneus region surviving multiple comparison corrections. These regions have previously been shown to be associated with decline from MCI to AD in

multiple studies as described above and are typical of an AD-type perfusion abnormality (see previous Chapters). In addition to decreased perfusion in the parietal region, we also found significantly reduced perfusion in the superior frontal lobe for decliners compared to non-decliners, predominant in the medial frontal supplementary motor area. Worse frontal perfusion in the insula, middle frontal and anterior cingulate regions in MCI decliners when compared to stable MCI has previously been identified (168), with left prefrontal and frontal areas showing high sensitivity and specificity for prediction of decliners (101). Abnormal perfusion in the supplementary motor area has also been found in an amnestic MCI sample when compared to normal controls (92). Although the clinical sample used in our analysis was unselected for diagnosis, it is likely that the SPM results are biased towards an Alzheimer's disease type pattern, due to the higher prevalence of AD type dementia compared to other subtypes. The use of a group analysis may also mask hypoperfusion in non-typical AD areas, for example in the frontal lobe, that may be present in some individuals in the decline group.

This study has limitations. The sample size is modest and due to small group sizes in regional patterns further validation in a large sub-group analysis is required. Additionally, we acknowledge that many factors such as depression or apathy, social influencers and worsening of other co-morbidities can affect dementia severity throughout follow up (254–258), however these influencers were not included in the analysis presented. Finally, the use of the CDR scale to measure dementia severity may not be optimal to determine patient decline in some dementia subtypes where memory is not the predominant symptom, such as in primary progressive aphasia. A combined outcome consisting of both dementia severity as measured by the CDR and a cognitive screening instrument such as the mini-mental state examination (MMSE) or Alzheimer's disease assessment scale cognitive subscale (ADAS-Cog) may provide a more rounded measure of overall dementia decline. Unfortunately, we were unable to obtain clinical scores for cognitive tests such as the MMSE for individuals in this study. Despite this, we found a good conversion rate for individuals with abnormal imaging (45% reached pre-defined outcome), with a decline rate comparable to that seen in a long-term follow-up study of AD individuals (252), providing a large separation in decline from those with normal imaging (18% reached pre-defined outcome). A longer follow-up period in addition to a

larger sample may however provide further differentiation between decline rates of pattern groups.

In conclusion, this study shows that perfusion SPECT imaging completed as part of clinical care at the time of diagnosis can identify individuals who are likely to decline and may be able to provide extra information on decline that is currently not being utilised. Individuals with poor rCBF on baseline SPECT imaging are at higher risk of faster decline than individuals with marginal or normal imaging and can be managed accordingly. Preliminary analysis suggests regional patterns of abnormality may show differing rates of decline and survival and could potentially be used to further discriminate individuals. Pattern decline rates however need to be validated in a larger sample with a longer follow up.

Chapter 6: Imaging care requirements

6.1 Introduction

Caregiver burden refers to the adverse effects perceived or experienced in caring for another person and may include psychological or physical feelings of stress, pressure, or fatigue. Factors found to increase caregiver burden in dementia include behavioural disturbances and patient apathy (259–261), patient dysfunction in memory and recognising emotions (262), and dementia severity (263). The dementia sub-type of frontotemporal dementia (FTD), in particular the behavioural variant, is particularly linked with high caregiver burden (263,264).

Identification of caregiver burden is important, with burden associated with depression and decreased quality of life for caregivers and with dementia patient institutionalisation (260,265,266). If burden is identified, implementation of caregiver and patient interventions have the potential to reduce caregiver burden (267), improve caregiver depression (268,269), delay patient institutionalisation (270,271) and improve caregiver quality of life (269,272).

The use of regional imaging to predict care needs and caregiver burden in a clinical setting is not well studied. Demonstration that abnormality within specific regions on imaging predicts caregiver burden, irrespective of dementia subtype or formal diagnosis, opens the way to better targeting of interventions for those at high risk of caregiver strain. This study aimed to investigate the relationship between caregiver burden and regional functional deficits on perfusion imaging.

6.2 Hypothesis

1. Patient rCBF on HMPAO SPECT can be used to predict caregiver burden as identified by the Zarit Burden Interview questionnaire.

6.3 Contributions and Collaborations

The study was conceived and designed by the author, Dr J. Spreadbury and Dr C. Kipps. Participants were recruited by the author and Ms S. Varkonyi through the BraIID study, with all participants undergoing HMPAO SPECT imaging at UHS. CDR questionnaires were completed by telephone with the consented patient's companion by a trained researcher (the author or Ms S. Varkonyi). Ethical approval for the BraIID study was obtained by the author. Initial image processing was performed by Dr L. Tossici-Bolt, Dr S. Michopoulou and Mr E. Varzarkis. All subsequent data processing and analyses was performed by the author. Interpretation of the data and preparation of tables and figures was carried out by the author.

6.4 Methods

6.4.1 Participants

Participants involved in the study gave written informed consent to join the Research Ethics Committee approved Brain Imaging in Dementia Study (BraIID). All patient participants from the BraIID study had reported cognitive decline at the time of recruitment and underwent perfusion HMPAO SPECT imaging as part of the diagnostic process. Companions of patients were also recruited to provide information on patient and caregiver characteristics.

Data from seventy-seven consecutive patients recruited to the BraIID study at the time of perfusion SPECT imaging, with a companion who knew them in a personal capacity, was analysed.

6.4.2 Demographics and neuropsychological data

Demographic information was collected from patient participants at the time of consent. Companions of patients completed the 22-item Zarit Burden Interview questionnaire (197) and an informant rated CDR (193), CBI (194,195) and FAQ (196) within two weeks of patient scanning. Further details on the questionnaires obtained is available in Chapter 2.

6.4.3 Perfusion SPECT imaging

Perfusion SPECT images were acquired on an INFINIA 3/8 HK4 dual head gamma camera, with parameters of acquisition and reconstruction outlined in Chapter 2 (Methods). In short, the camera was equipped with low energy high resolution collimators, following a circular orbit with radius minimised for each patient. The matrix size was 128x128 with a pixel size of 3.32mm. Images were checked for movement and reconstructed using a filtered back projection method with scatter correction.

In preparation for statistical analysis, the HMPAO SPECT reconstructed images were spatially normalised to a standard structural template and smoothed with a 16mm kernel using statistical parametric mapping 8 (SPM8) (38). Cerebellar normalisation was also completed using in-house MATLAB code, correcting for age. Further details are available in Chapter 2 (Methods).

Regions of interest (ROI) for the frontal, temporal and parietal lobes were created using WFU-pickatlas software and the Automated Anatomical Labelling digital (AAL) atlas (206-208). Average normalised voxel value counts were obtained for each region of interest using the SPM8 marsbar toolbox for both patients and controls, giving a quantitative indication of perfusion (209). Perfusion ROIs for each individual were classified as normal or abnormal using a two standard deviation cut-off from 31 age matched control image ROIs (see Chapter 2).

6.4.4 Statistical analysis

The ZBI score was divided into those with little or no burden (score 0-20), mild to moderate burden (score 21-40) and moderate to severe and severe burden (score 41-88) as per recommended ZBI cut-offs. Moderate to severe and severe burden groups were combined due to small numbers of individuals in the severe burden group ($N = 3$).

SPM8 voxel based imaging comparisons of burden groups with controls to identify patterns of abnormally reduced perfusion were completed. Mann-Witney U tests were used to compare mean Zarit burden score across the same brain regions of interest. Correlation analysis compared ZBI total score and perfusion values within regions of interest. Follow-up exploratory analyses

using Spearman's rho ($p < .01$ uncorrected threshold) tested for associations between specific ZBI items and regional perfusion values to identify whether specific regional deficits were associated with particular profiles of caregiver burden. The influence of age, patient and caregiver gender, CDR sum of boxes score, CBI total and domain score and FAQ score on caregiver burden was also assessed using Chi-square and Kruskal-Wallace testing. CBI score for each domain and total score was converted into a percentage of impairment: 0-25% was classified as mild, 26-50% as moderate, 51-75% as severe, and more than 75% as very severe. This grading system was based on studies that have investigated the CBI tool (195,273).

6.5 Results

6.5.1 Sample demographics

Patient demographics are shown in Table 6-1. Caregivers in our sample were mostly female (71%) and a spouse or partner of the patient with cognitive complaints (80%), although some were siblings (8%), adult children (8%), and friends (4%). Caregivers had a median ZBI burden score of 23, ranging from 0 to 72 points out of 88. There were slightly more male patients than female (65%), with a median caregiver rated clinical dementia rating score of 0.5 and median functional activity score of 9 out of 30. The mean age of patients was 69 years old (SD = 8.34). The majority of patient participants were living with their spouse or partner (88%) and were retired or not working due to illness (84%), although a small percentage were still working at the time of recruitment (10%; 6% working status unknown). Most individuals were right-handed (89%) with a small number left-handed (7%; 4% unknown). Burden score was not related to either caregiver or patient gender, however burden groups differed significantly with respect to age (χ^2 (2, N = 77) = 10.33, p = .006). On average, those with none to little burden were younger than those with mild to moderate or moderate to severe burden. Age, however, did not appear to influence perfusion group status (normal versus abnormal).

Table 6-1 Sample demographics

Cohort demographics grouped by Zarit burden interview questionnaire into little or no burden, mild to moderate burden, and moderate to severe burden. Abbreviations: M, Male; F, Female; R, right; L, left. * Total FAQ *N* = 71, CDR *N* = 61

	Little or no burden	Mild to moderate burden	Moderate to severe burden	Statistics (*p*-value)
N	31	32	14	-
Patients M/F	16/15	24/8	10/4	*ns*
Companions M/F	12/19	7/25	3/11	*ns*
Patient handedness R/L (Unknown)	29/1 (1)	28/4	12 (2)	*ns*
Patient age in years (mean ± S.D.)	65.6 ± 8.36	72.2 ± 8.25	69.8 ± 5.54	.006
Patient global CDR (median with range)*	0.5 (0 – 1)	0.5 (0.5 – 3)	1.0 (0.5 – 2)	.006
Patient FAQ score (mean ± S.D.)*	7.27 ± 1.23	12.0 ± 1.09	13.9 ± 2.55	.005

6.5.2 Associations between caregiver burden group and patient regional abnormality

When comparing ZBI burden groups against controls on a voxel by voxel basis using statistical parametric mapping (Figure 6-1), burden score was shown to be strongly associated with global brain dysfunction, with global perfusion decreasing with increasing burden group (Table 6-2). In addition, burden was also associated with specific regional brain dysfunction. Caregivers with moderate to severe burden showed a predominantly frontal and right hemisphere reduction in perfusion when compared to controls. Those with mild to moderate burden showed a main area of reduced function in the left temporoparietal region around the angular gyrus, with generalised reduced function across the rest of the brain.

A Mann-Whitney test comparing regions and burden indicated that ZBI total score was higher for individuals with abnormal frontal lobe perfusion (left frontal: U = 408.5, $p < .05$; right frontal: U = 491.0, $p < .05$) than those with normal perfusion in these regions. Total ZBI score was worse in those with right parietal (U = 500.5, $p < .05$) and right temporal (U = 431.5, $p < .01$) abnormal perfusion. No difference was seen in total ZBI between abnormal and normal left hemisphere parietal and left hemisphere temporal region groups.

Table 6-2 Burden group regional imaging z-scores

Imaging region of interest z-scores for each burden group when compared to perfusion SPECT controls. Burden grouping was completed using the Zarit burden interview questionnaire.

	Global	Frontal		Parietal		Temporal	
		Left	Right	Left	Right	Left	Right
Little to no burden	-0.92	-0.85	-0.92	-1.10	-1.02	-0.92	-0.74
Mild to moderate burden	-1.82	-1.42	-1.53	-1.96	-1.88	-1.78	-1.73
Moderate to severe burden	-2.10	-2.18	-2.42	-1.98	-2.20	-1.55	-1.84

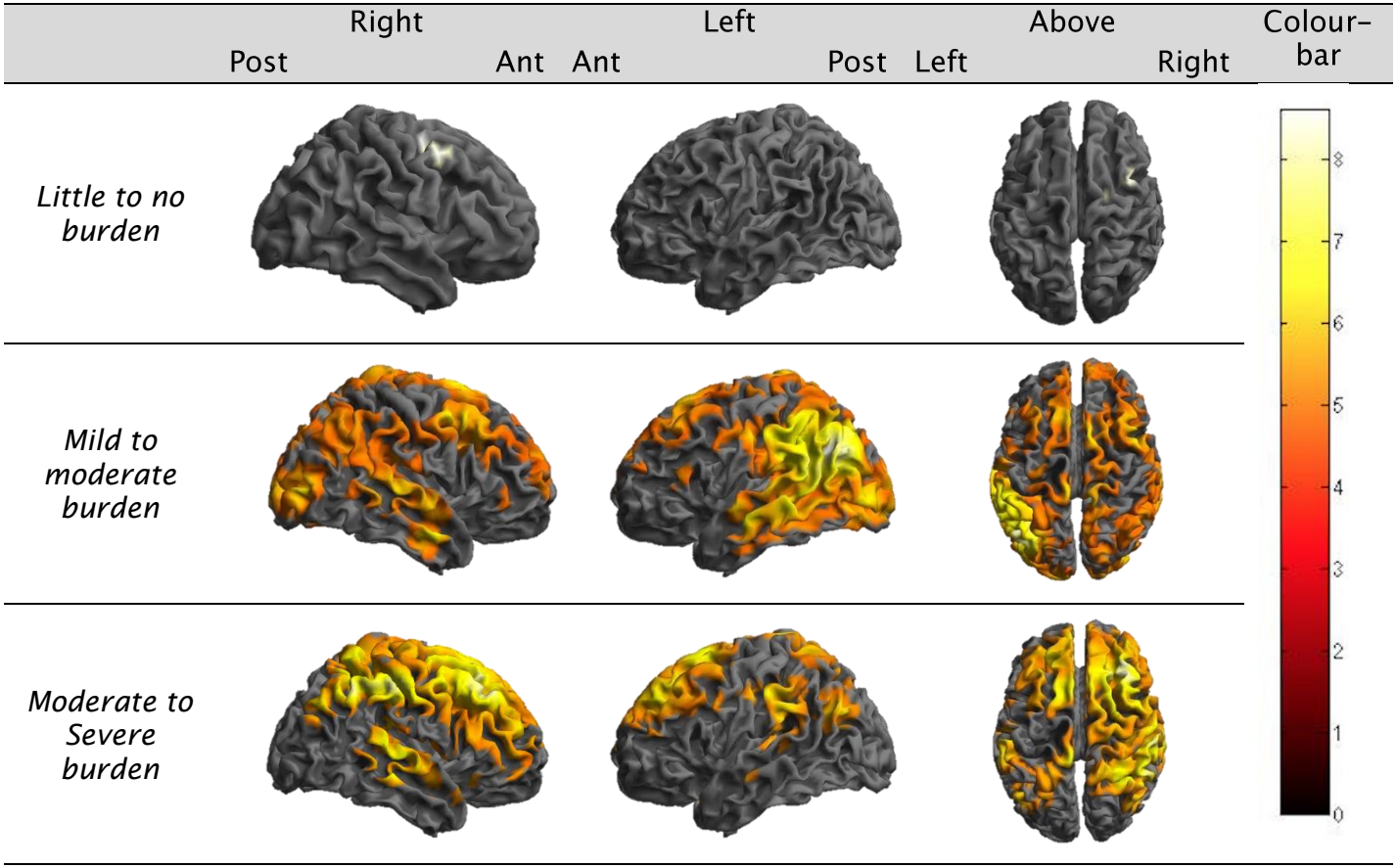


Figure 6-1 SPM comparisons of burden groups compared to controls

Areas of reduced perfusion (heat scale) in individuals with little to none, mild to moderate, and moderate to severe burden as measured by the ZBI questionnaire when compared to controls ($p < .05$ FWE corrected). Ant, anterior; Post, posterior.

6.5.3 Correlations between burden score and perfusion values

Spearman's rho correlations between ZBI total score and cortical regions showed negative correlations indicative of higher caregiver burden being associated with lower perfusion scores. The ZBI total score correlated negatively and significantly with both left (r_s = −.337, p = .003) and right (r_s = −.369, p < .001) frontal regions, left (r_s = −.304, p = .007) and right (r_s = −.313, p = .006) parietal regions, and temporal right (r_s = −.336, p = .003) hemisphere only region. The left temporal region was not significantly correlated (r_s = −.217, p = .058).

Exploratory correlation analysis between individual item-region correlations revealed both item generalisation and specificity (all p < .01, Table 6-3). Items that significantly correlated with perfusion scores across all regions included those measuring stress and impact on health. In contrast, items measuring patient demands, personal time constraints, privacy and social life were specific to the frontal regions, with right sided dominance. Frontal and right temporal regions also correlated significantly with embarrassment and dependency.

Table 6-3 Correlation analysis between rCBF and ZBI items

Exploratory correlations between individual Zarit burden items and regional HMPAO SPECT perfusion values. Correlations significant at the $p < .01$ level are highlighted. L, left; R, right. The wording of some items has been condensed for brevity.

		Frontal		Parietal		Temporal	
		L	R	L	R	L	R
Zarit item 1 – Do you feel that your relative asks for more help than they need?	r_s	−0.298	−0.260	−0.202	−0.207	−0.217	−0.215
	Sig.	.007	.020	.072	.065	.053	.056
Zarit item 2 – Do you feel that because of the time you spend with your relative that you don't have enough time for yourself?	r_s	−0.268	−0.314	−0.230	−0.250	−0.150	−0.230
	Sig.	.016	.005	.041	.025	.183	.040
Zarit item 3 – Do you feel stressed between caring for your relative and trying to meet other responsibilities?	r_s	−0.377	−0.442	−0.460	−0.472	−0.298	−0.403
	Sig.	.001	< .001	< .001	< .001	< .001	< .001
Zarit item 4 – Do you feel embarrassed over your relative's behaviour?	r_s	−0.412	−0.389	−0.264	−0.223	−0.267	−0.299
	Sig.	< .001	< .001	.018	.047	.017	.007
Zarit item 5 – Do you feel angry when you are around your relative?	r_s	−0.125	−0.097	−0.273	−0.227	−0.185	−0.161
	Sig.	.269	.391	.014	.043	.100	.153
Zarit item 6 – Do you feel that your relative affects your relationships with others in a negative way?	r_s	−0.140	−0.194	−0.104	−0.165	0.007	−0.180
	Sig.	.217	.084	.359	.145	.948	.109
Zarit item 7 – Are you afraid what the future holds for your relative?	r_s	−0.141	−0.234	−0.131	−0.205	−0.081	−0.264
	Sig.	.213	.036	.246	.068	.476	.018
Zarit item 8 – Do you feel your relative is dependent on you?	r_s	−0.234	−0.260	−0.211	−0.222	−0.223	−0.350
	Sig.	.037	.020	.061	.047	.047	.001

Table 6-3 Correlation analysis between rCBF and ZBI items (cont.)

		Frontal		Parietal		Temporal	
		L	R	L	R	L	R
Zarit item 9 – Do you feel strained when you are around your relative?	r_s	−0.316	−0.302	−0.318	−0.303	−0.233	−0.272
	Sig.	.004	.006	.004	.006	.037	.015
Zarit item 10 – Do you feel your health has suffered because of your involvement with your relative?	r_s	−0.311	−0.348	−0.344	−0.339	−0.276	−0.307
	Sig.	.005	.002	.002	.002	.013	.006
Zarit item 11 – Do you feel that you don't have as much privacy as you would like because of your relative?	r_s	−0.216	−0.290	−0.224	−0.273	−0.116	−0.205
	Sig.	.054	.009	.046	.014	.306	.069
Zarit item 12 – Do you feel that your social life has suffered because you are caring for your relative?	r_s	−0.211	−0.290	−0.245	−0.278	−0.153	−0.286
	Sig.	.061	.009	.028	.013	.174	.010
Zarit item 13 – Do you feel uncomfortable about having friends over because of your relative?	r_s	−0.176	−0.258	−0.108	−0.161	−0.052	−0.196
	Sig.	.119	.021	.340	.155	.647	.082
Zarit item 14 – Do you feel that your relative expects you to take care of them as if you were the only one they could depend on?	r_s	−0.319	−0.338	−0.242	−0.282	−0.178	−0.275
	Sig.	.004	.002	.033	.013	.119	.015
Zarit item 15 – Do you feel that you don't have enough money to take care of your relative?	r_s	0.031	−0.016	0.101	0.053	0.153	0.027
	Sig.	.788	.890	.376	.645	.179	.810
Zarit item 16 – Do you feel that you will be unable to take care of your relative much longer?	r_s	−0.178	−0.199	−0.106	−0.108	−0.092	−0.093
	Sig.	.117	.078	.353	.344	.418	.414

Table 6-3 Correlation analysis between rCBF and ZBI items (cont. 2)

		Frontal		Parietal		Temporal	
		L	R	L	R	L	R
Zarit item 17 – Do you feel you have lost control of your life since your relative's illness?	r_s	−0.202	−0.242	−0.209	−0.212	−0.114	−0.236
	Sig.	.074	.032	.064	.061	.317	.036
Zarit item 18 – Do you wish you could leave the care of your relative to someone else?	r_s	−0.228	−0.180	−0.105	−0.071	−0.097	−0.006
	Sig.	.044	.112	.355	.533	.394	.959
Zarit item 19 – Do you feel uncertain about what to do about your relative?	r_s	−0.180	−0.191	−0.084	−0.127	−0.065	−0.222
	Sig.	.113	.091	.461	.264	.569	.049
Zarit item 20 – Do you feel you should be doing more for your relative?	r_s	0.084	0.033	−0.025	−0.059	0.024	−0.119
	Sig.	.461	.770	.826	.604	.832	.294
Zarit item 21 – Do you feel you could do a better job in caring for your relative?	r_s	0.085	0.067	−0.012	−0.024	0.053	−0.028
	Sig.	.455	.556	.918	.835	.646	.804
Zarit item 22 – Overall, how burdened do you feel in caring for your relative?	r_s	−0.242	−0.226	−0.161	−0.122	−0.194	−0.200
	Sig.	.032	.045	.156	.283	.086	.078
Zarit total score	r_s	−0.337	−0.369	−0.304	−0.313	−0.217	−0.336
	Sig.	.003	.001	.007	.006	.058	.003

6.5.4 Influencing covariates

CDR global score indicating dementia symptom severity was significantly different between burden groups (H(2) = 10.3 , p = .006), and CDR showed a moderate positive correlation to burden score (r_s = 0.355, p < .005). Partial rank correlations correcting for CDR in the relationship between burden and regional perfusion showed significance remained for the right frontal (r_s = −.273, p = .035), left (r_s = −.283, p = .028) and right (r_s = −.317, p = .014) parietal, and right temporal (r_s = −.376, p = .003) regions.

Burden groups also differed on Functional Activities Questionnaire performance, a specific measure of a patient's dependence and social function (instrumental activities of daily living). As burden increased, FAQ worsened (H(2) = 10.0, p = .007), with FAQ showing a moderate positive correlation to burden score (r_s = 0.434, p < .001). This appeared to be driven by the right frontal region only, with a higher FAQ seen in those with reduced perfusion in this region (p = .037). Partial rank correlations between burden and regions correcting for FAQ showed weak significant correlations between burden and right frontal (r_s = −.250, p = .037), right parietal (r_s = −.248, p = .039), and right temporal (r_s = −.251, p = .036) regions remained.

Comparison of behavioural score as measured by the CBI showed a significant relationship between total score and burden (χ^2 (6, N = 76) = 24.70, p < .001), with higher behavioural severity score associated with worse caregiver burden. When individual domain impairment groups were compared to burden groups, motivation (χ^2 (6, N = 76) = 26.16, p < .001), skills (χ^2 (6, N = 77) = 17.09, p < .01), mood and stereotypic and motor behaviour domains (χ^2 (6, N = 77) = 16.721, p < .01) showed strong associations with burden.

6.6 Discussion

The use of perfusion SPECT imaging for diagnosis is well established, but there is further information available from scans that is scarcely used and which may be valuable for assessing care needs. In this study we demonstrated that regional perfusion values on HMPAO SPECT scans were strongly predictive of caregiver burden, irrespective of diagnosis. We found that burden is not only

associated with the global pathological load but also with the pattern of regional pathology seen on imaging (Figure 6-1).

Caregiver burden was largely driven by frontal lobe dysfunction. Those with reduced left or right frontal perfusion had a significantly higher burden score than those with normal frontal perfusion. This is likely due to patient behavioural or personality changes (neuropsychiatric symptoms), with higher caregiver burden associated with worse patient behavioural scores. Such neuropsychiatric features are well documented for behavioural variant frontotemporal dementia, with patients frequently showing disinhibition, selfishness, a lack of insightfulness, a loss of embarrassment and irritability and aggression (274). Of note, however, is that we did not stratify the analysis by diagnosis, but analysed burden simply in relation to regional pathology. A relationship between frontal pathology and neuropsychiatric symptoms is also present in other dementia subtypes, including Alzheimer's disease (275–281). Behavioural symptoms are frequently identified as a significant contributor to caregiver burden (282,283).

Greater caregiver burden was also predicted by dysfunction of the right parietal and temporal regions. The right hemisphere has been demonstrated to be involved in mediating social and emotional behaviour in a mixed clinical sample previously in both structural (284–286) and functional imaging studies (287–289), with right sided changes associated with delusions, disinhibition, irritability, elation and sleep disorder (290).

Although any reduction in cortical function correlated with an increase in caregiver burden, post-hoc ZBI item analysis suggested that frontal type perfusion abnormality, predominantly right-sided, may place burden on caregivers through high patient demands, reduced privacy and social life for the caregiver, in addition to increased caregiver time constraints. Those with either frontal or right temporal regional functional reduction may predispose to higher levels of stress by exhibiting embarrassing behaviour and engendering higher levels of dependency. This information could be used to better target caregiver interventions to prevent or reduce caregiver burden at the time of diagnosis and not simply when problem behaviours contribute to crisis.

It has previously been noted that clinical dementia rating (CDR) score correlates strongly to caregiver burden, with increased dementia severity associated with higher caregiver burden scores (263). Similar correlations between caregiver burden and patient functional activities of daily living have also been found (291). Our results agree with these findings, with overall CDR and FAQ higher in those with greater caregiver burden. Patient dementia severity and function however had little influence on the relationship between caregiver burden and patient regional perfusion deficits, suggesting that the location of reduced function affects burden independently from patient disease stage.

Previous studies highlight an importance for distinguishing the diagnostic subtypes of dementia when investigating caregiver burden and reactions to problems (292). Although clearly inter-related, our results suggest that caregiver burden is predicted by regional abnormality, independent of a confirmed dementia sub-type diagnosis. While regional abnormality largely defines the subsequent dementia syndrome, symptoms in dementia reflect location of underlying pathology. Frontal features are typical of FTD but are largely underappreciated in AD, despite their potential to impact caregiver burden. Although in general caregivers of FTD patients experience more burden than those with AD (292), simply grouping patients by subtype to identify caregivers at risk of high burden might fail to identify individuals with AD who have frontal or right predominant imaging deficits that may potentially present with challenging or complicated behaviours. Neuropsychiatric symptoms are common in AD patients (293–295), mild cognitive impairment groups (296–298) and are also frequently seen in mixed type and vascular dementia (299–301).

There are potential limitations to this study. Caregiver burden is multifactorial, and we did not control for the full range of potential confounding variables. In particular, we did not have measures of caregiver depression, which has been shown to be a strong influencer of burden, particularly in adult children caregivers (302). Other factors such as hours providing care and level of support provided by health services can also affect burden but were not measured in this study. These factors can however be measured directly in individual cases, and our findings suggest an influence of region-specific changes that are independent of caregiver characteristics on caregiver burden.

In conclusion, regional abnormality on perfusion SPECT imaging at the time of diagnosis can be used to identify individuals likely to place the highest burden on their caregivers. This study contributes further understanding by identifying a component of caregiver burden that is independent of caregiver characteristics, and emphasises those features of burden that are most influenced by underlying pathology. Burden is most affected by frontal, or right-predominant parietal and temporal lobe dysfunction, irrespective of dementia subtype. With multidimensional caregiver interventions being effective in delaying patient institutionalisation and improving caregiver quality of life, this knowledge can be used to intervene with targeted support for caregivers with highest risk.

Chapter 7: The clinical utility of perfusion SPECT imaging

7.1 Introduction

With the development of statistical methods of imaging analysis (such as VBM), perfusion SPECT imaging has been shown to have improved diagnostic accuracy over clinical assessment and structural imaging alone (191,303). Semi-quantitative methods of analysis have also been shown to provide a significant increase in diagnostic accuracy and inter-rater reliability over visual assessment (22,35,36,76,303). Despite the benefits of semi-quantitative analysis, it is rarely used clinically to aid reporting. Additionally, although there is extensive literature on perfusion SPECT imaging accuracy in dementia diagnosis, research has been limited as to its actual ability to change diagnosis and alter diagnostic confidence in clinical situations. Prior studies investigating the influence of perfusion SPECT imaging on diagnosis and diagnostic confidence in clinical samples are now outdated in methods of acquisition, analysis and reporting (57,129,304), and we are unaware of any recent studies exploring the utility of the technology to alter diagnostic confidence using semi-quantitative methods. Clinician opinion on the usefulness of perfusion SPECT using individual against control group SPM analysis and reporting in aiding dementia diagnosis is also unknown, despite this being recently investigated in other imaging modalities such as DaTSCAN imaging, FDG PET, amyloid PET, and MRI hippocampal atrophy (305–309).

Knowledge of the utility of perfusion SPECT imaging is essential to determine whether the technology provides added value beyond standard clinical workup and to provide evidence for its continued use in dementia diagnosis. This study aimed to assess the impact of perfusion SPECT imaging on the diagnostic process and determine its clinical usefulness using semi-quantitative methods of analysis for the individual. Clinician opinion on perfusion SPECT imaging in aiding the diagnostic process was also investigated.

7.2 Hypothesis

Perfusion SPECT imaging contributes to the diagnostic process, by:

1. influencing diagnosis in a clinical sample of individuals with cognitive impairment and suspected dementia
2. improving diagnostic confidence in referring clinicians

7.3 Contributions and Collaborations

The study was conceived and designed by the author and Dr C. Kipps. The questionnaires were developed by the author and sent out to referring clinicians by Ms M. Bright of the UHS Nuclear Medicine administration team. Image analysis and reporting was completed externally to this study as part of normal clinical care and images in this chapter were not directly analysed by the author. Data collation, statistical analysis, interpretation of the data and preparation of tables and figures was carried out by the author.

7.4 Methods

7.4.1 Sample

Clinicians who referred patients with cognitive complaints to University Hospital Southampton NHS Foundation Trust (UHS) for a perfusion SPECT scan between November 2015 and September 2017 were assessed by questionnaire. All patients were referred for a HMPAO SPECT scan due to diagnostic doubt at the time of scanning. Questionnaires were sent to all clinicians referring for perfusion SPECT imaging across the Wessex region in the South of England. As the questionnaires were developed as service improvement and evaluation tools, the study was deemed clinical service improvement and did not directly involve patient participants. Clinician consent was implied with return of the questionnaires.

7.4.2 Perfusion SPECT imaging and analysis

Perfusion SPECT images were acquired on a GE INFINIA 3/8 HK4 dual head gamma camera as per normal clinical care, with parameters of acquisition and reconstruction outlined in Chapter 2 (Methods). In preparation for statistical analysis, the HMPAO SPECT reconstructed images underwent spatial normalisation, smoothing and cerebellar normalisation as per the SPM pre-processing procedure described in Chapter 2 (Methods).

For image analysis, single-subject voxel based hypoperfusion maps were created for each individual when compared to 31 SMV/INFINIA camera age-matched controls using statistical parametric mapping. A significance threshold of $p < .001$ (uncorrected for multiple comparisons) with 100 voxel cluster threshold was used. Image analysis was also carried out using an automated 3D surface projections (3D-SSP) software by EXINI diagnostics (brain), with further details detailed in Chapter 2 (Methods) and Hagerstrom et al (210).

Images were read based on both the SPM maps and 3D surface projections by a consultant physician experienced in reporting perfusion SPECT images (Dr C. Kipps). Perfusion SPECT scans were interpreted as either normal or likely neurodegenerative and suggestive of Alzheimer's disease, frontotemporal dementia, dementia with Lewy bodies, vascular, or other dementia. Patterns of perfusion reported were based on clinical expertise and current literature.

7.4.3 Assessment of clinical impact

To assess the impact of the perfusion SPECT imaging on clinician diagnosis and diagnostic confidence, clinicians were assessed by questionnaire both before and after patient imaging. At the time of imaging referral (pre-SPECT imaging), the referring clinician was sent an invitation to participate letter, an initial pre-imaging questionnaire (see Appendices), the original referral request for the patient and a stamped addressed return envelope. The clinician was asked to indicate their provisional diagnosis for the patient from a list of diagnoses, confidence in that diagnosis ranked on a 100mm visual analogue scale (VAS) ranging from not confident at all (0mm) to extremely confident (100mm) and up to three other potential diagnoses. Diagnoses given were: Alzheimer's disease, Frontotemporal dementia, Vascular dementia, Dementia

with Lewy bodies, Mild cognitive impairment, Psychiatric, Dementia – other and Neurology – other. A space was left for detail to be added if Dementia – other or Neurology – other was chosen. After completion, the clinicians were asked to return the questionnaire in a stamped addressed envelope which was included in the original letter.

After imaging was completed and reported, the imaging report was sent back to the referring clinician with a second questionnaire (see Appendices). Clinicians were asked again to indicate their provisional diagnosis from the same list as previously, confidence in diagnosis and other potential diagnoses. To determine the perceived usefulness of the perfusion SPECT scan, they were asked to indicate:

- how helpful they thought the imaging was in aiding their diagnosis on a 100mm VAS scale from not helpful at all (0mm) to extremely helpful (100mm)
- whether they thought the imaging contributed to their diagnostic process (binary yes/no)
- whether they thought the imaging improved, did not contribute to, or confused their understanding of the patient's disease

Clinicians were not informed of their pre-imaging questionnaire answers at the time of completing the post-imaging questionnaire. The clinician then returned the questionnaire in a stamped addressed envelope included in the letter.

To remove the risk of order effects in the questionnaires, six versions of each referrer questionnaire were produced where diagnostic options were reordered. Questionnaires were subsequently sent out in order of questionnaire version (A, B, C, D, E, F) to ensure order bias was minimised.

7.4.4 Statistical analysis

Descriptive analysis was completed to assess sample demographics and questionnaire variables before and after perfusion SPECT imaging. Paired t-tests and ANOVA were used to determine change in diagnostic confidence between pre- and post-imaging questionnaire for both all individuals and by clinician pre-imaging confidence divided into very low (0-25mm), low to

moderate (26-50mm), moderate to high (51-75mm) and very high (76-100mm) confidence.

To assess the influence of perfusion SPECT imaging on change in diagnosis, both provisional diagnoses and SPECT result were grouped into neurodegenerative (AD, DLB, FTD or dementia-other) or non-neurodegenerative (normal, vascular, psychiatric or neurology-other) and compared.

Due to the inability to distinguish whether a diagnosis of mild cognitive impairment (MCI) was stable or progressive, results were examined both including and excluding those diagnosed with MCI.

7.5 Results

7.5.1 Questionnaires

In total, 212 pre-imaging questionnaires and 141 post-imaging questionnaires were sent to clinicians during the service evaluation. Of the pre-imaging questionnaires, 147 questionnaires were returned (74% completion rate), with 13 scans cancelled before the return of the questionnaire. Eight patients had their imaging cancelled after return of the pre-imaging questionnaire. After those with cancelled scans were removed, a total of 139 returned pre-imaging questionnaires remained. Of the post-imaging questionnaires, 89 were returned (63% completion rate), with 80 of these having matching pre-imaging questionnaires. One of these was deemed invalid due to the pre-imaging and post-imaging questionnaires being completed by different clinicians. A total of 79 matching valid questionnaires remained for the analysis.

The pre-imaging questionnaire was received on average 15.3 days (mean, S.D. = 20.8) before imaging, with the post-imaging questionnaire received on average 40.7 days (mean, S.D. = 23.5) after imaging was completed.

7.5.2 Sample

7.5.2.1 Clinicians

The 79 matching questionnaires were returned by 41 clinicians, consisting of 9 neurologists, 30 psychiatrists, and 2 stroke physicians. All referring clinicians were either at consultant or associate specialist level at the time of completion of the questionnaire.

7.5.2.2 Patients

The age of patients at time of scanning ranged from 43 to 90 years old (mean = 66.8, S.D. = 10.7), with the sample consisting of 39 males and 40 females. The pre-imaging diagnoses for all patients were as follows: Alzheimer's disease (*N* = 29), Mild cognitive impairment (*N* = 14), Frontotemporal dementia (*N* = 15), Dementia with Lewy bodies (*N* = 2), Dementia – other (*N* = 7), Vascular (*N* = 2), Psychiatric (*N* = 5) and Neurology – other (*N* = 5).

7.5.3 Perfusion SPECT reports and change in diagnosis

Diagnosis between pre-imaging and post-imaging questionnaires changed in 49% of cases, which included changes between MCI and other diagnoses including specific dementia subtypes (e.g. MCI to AD or vice versa). Diagnosis change by pre-imaging provisional diagnosis for those with Alzheimer's disease and Frontotemporal dementia is shown in Figure 7-1. Excluding MCI cases, diagnosis changed after perfusion SPECT imaging in 25/57 (44%) of cases in this sample. Of these, 16 moved between a neurodegenerative and non-neurodegenerative diagnosis category.

When diagnoses were grouped into neurodegenerative and non-neurodegenerative categories (excluding MCI cases) and pre- and post-imaging diagnosis were compared, SPECT agreed with the pre-imaging diagnosis in 61% and disagreed in 39% of cases. When SPECT agreed with the pre-imaging diagnosis, diagnosis remained unchanged in 34/35 (97%) and changed in one case (3%). When SPECT disagreed with the pre-imaging diagnosis clinician diagnosis changed in 15/22 (68%) and remained unchanged in 7/22 (32%).

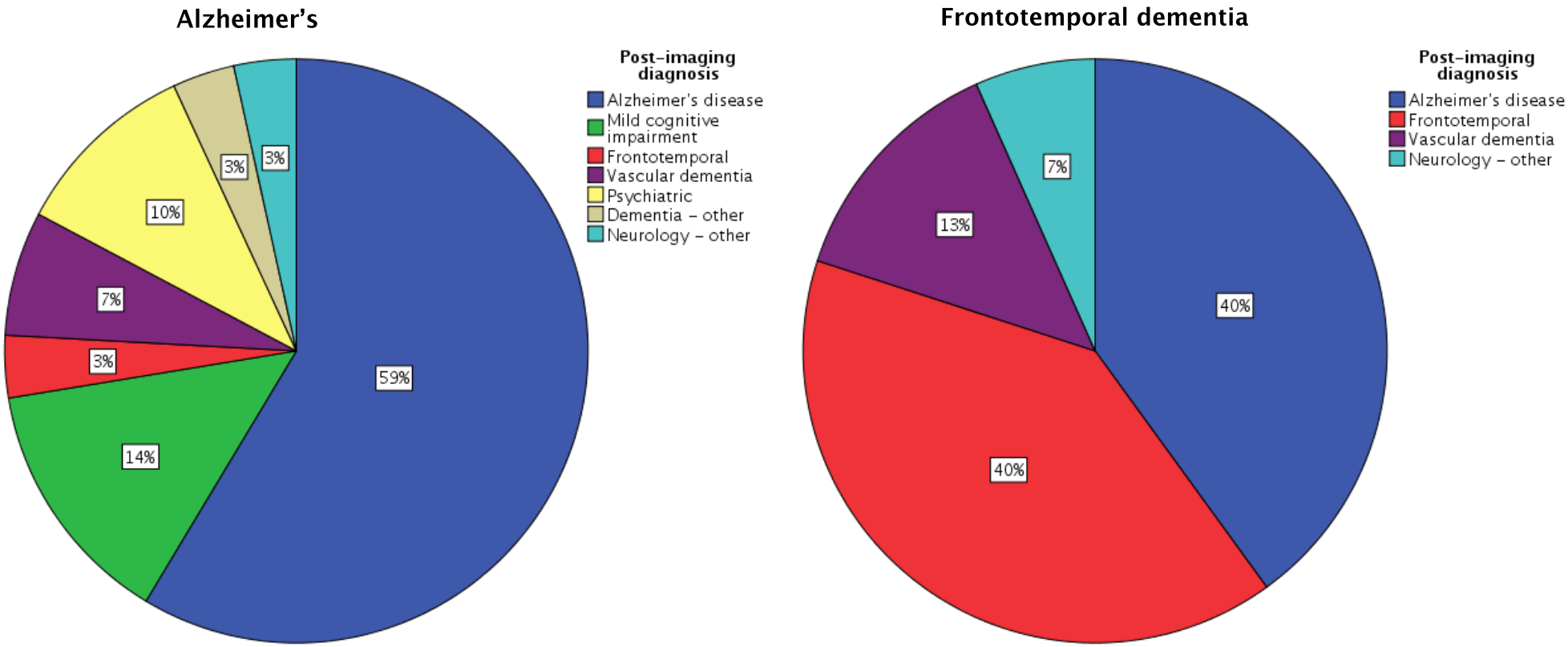


Figure 7-1 Change in diagnosis for Alzheimer's disease and frontotemporal dementia

Pie charts showing post-imaging diagnoses for participants with a pre-imaging diagnosis of Alzheimer's disease (left) and frontotemporal dementia (right). Percentages of total individuals are presented. Diagnosis changed after imaging in 41% of cases with a pre-imaging diagnosis of AD and 60% of cases with a pre-imaging diagnosis of FTD. *Percentages may not add up to 100% due to rounding.

7.5.4 Diagnostic confidence change

Diagnostic confidence as measured by VAS was unchanged between pre-imaging (mean = 57.2mm, S.D. = 18.9mm) and post-imaging (mean = 61.6mm, S.D. = 22.5mm) questionnaires when compared en masse using a paired t-test ($p > .05$).

When subjects were grouped by pre-imaging diagnostic confidence, clinician confidence was significantly increased (mean change = +26.3mm, S.D. = 22.2, $p < .001$) after imaging in cases where the pre-imaging confidence was low (<50mm on VAS). Clinician confidence was not significantly different ($p < .05$) after imaging when pre-imaging confidence was moderate to high (>50mm on VAS; mean change = -6.6mm, S.D. = 25.5). Those with very high pre-imaging confidence (>75mm on VAS) showed a slight reduction in confidence after imaging (mean change = -26.8mm, S.D. = 36.3, $p < .05$), with high variation in individual responses. Confidence changes can be seen in Figure 7-2 and Table 7-1.

There was no significant difference in diagnostic confidence change between clinician speciality groups ($F(2,76) = 1.68$, $p = .193$), nor between consultants and speciality doctors ($t(77) = 0.077$, $p = .939$). Clinicians were less diagnostically certain for male patients (mean = 51.9mm, S.D = 19.5) than female patients (mean = 62.4mm, S.D. = 17.0) before imaging ($p < .05$), however no significant difference was found in diagnostic confidence after imaging or change in diagnostic confidence between questionnaires ($p > .05$).

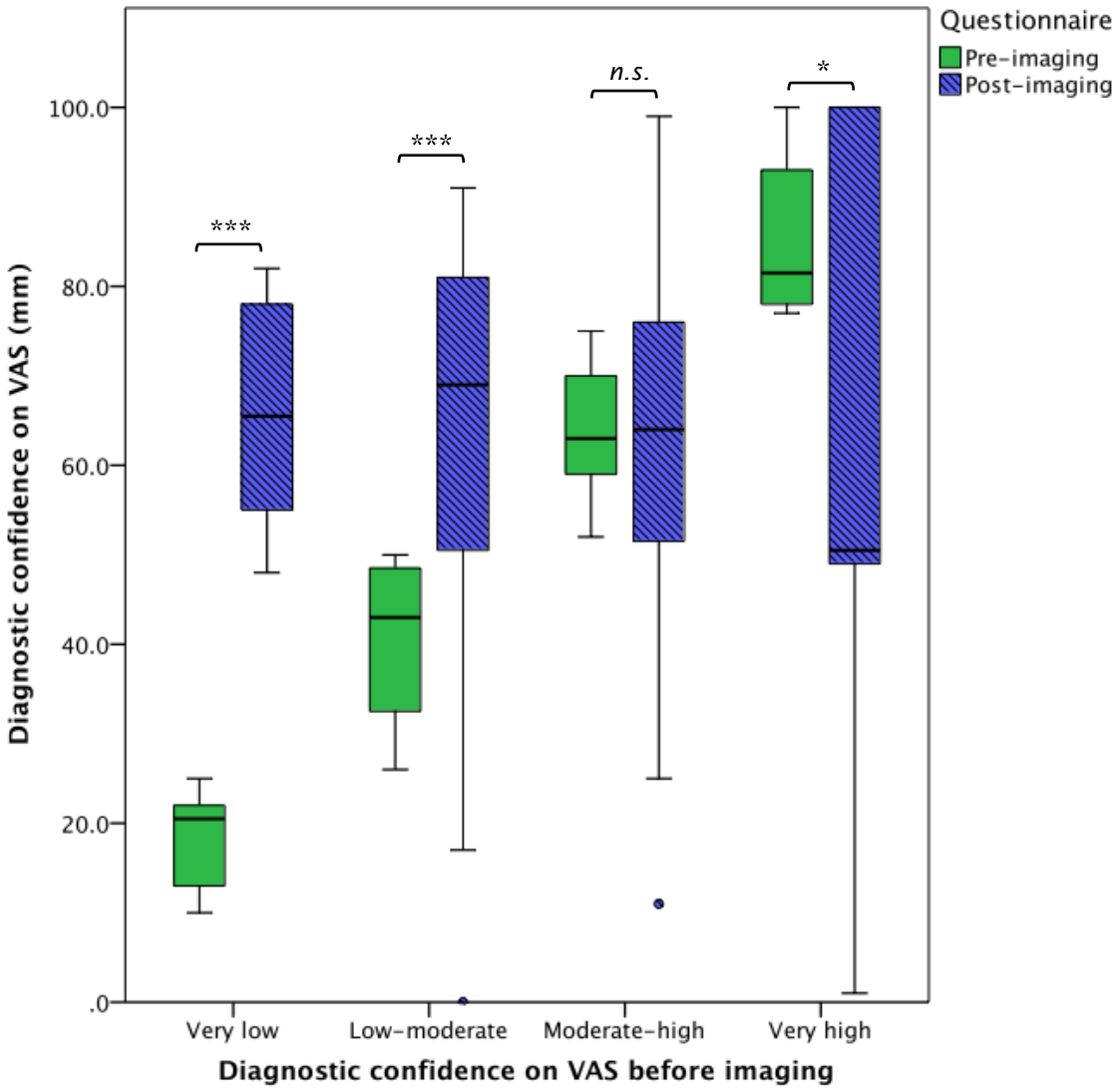


Figure 7-2 Diagnostic confidence change between pre- and post-imaging questionnaires

Box plot showing change in diagnostic confidence after perfusion SPECT imaging as measured by a VAS scale from 0mm (not confident at all) to 100mm (extremely confident), grouped by pre-imaging confidence into very low (0-25mm), low-moderate (26-50mm), moderate-high (51-75mm) and very high (76-100mm) confidence groups. *$p < .05$, ***$p < .001$, *n.s.* = not significant.

Table 7-1 Change in diagnostic confidence by pre-imaging confidence group

Change in diagnostic confidence as measured by a 100mm VAS scale from 0mm (not confident at all) to 100mm (extremely confident), grouped by pre-imaging confidence into very low (0-25mm), low-moderate (26-50mm), moderate-high (51-75mm) and very high (76-100mm) confidence groups.

Pre-imaging Confidence (mm)	*N*	Confidence change Mean ± SD (mm)	Statistics (*p* value)	
0-25	6	47.2 ± 14.5	.001	< .001
26-50	20	20.1 ± 20.3	.0002	
51-75	43	-1.8 ± 20.0	.545	.066
76-100	10	-26.8 ± 36.3	.045	

Table 7-2 High confidence clinicians

Information on individuals for whom clinicians had high diagnostic confidence (>75mm on VAS scale) before imaging. NAD = No appreciable disease, N.D. = Not diagnostic

ID	Diagnosis (pre-imaging)	Confidence (mm)	Diagnosis (post-imaging)	Confidence (mm)	Confidence change (mm)	SPECT result
1	FTD	81	FTD	100	19	FTD
2	AD	85	AD	100	15	AD
3	AD	100	AD	100	0	AD
4	AD	78	AD	74	-4	AD
5	MCI	77	MCI	51	-26	NAD
6	FTD	78	AD	50	-28	AD
7	AD	82	AD	49	-33	AD (N.D.)
8	AD	93	AD	49	-44	AD (N.D.)
9	AD	80	MCI	1	-79	NAD (N.D.)
10	MCI	93	AD	5	-88	NAD / Vascular

7.5.5 Clinician opinion

When clinicians were asked directly for their opinion of the imaging, 96% (*N* = 76) thought perfusion SPECT contributed towards the diagnostic process, while 4% (*N* = 3) said it did not contribute. On a VAS 100mm scale from 0mm (not helpful at all) to 100mm (extremely helpful), clinicians rated perfusion SPECT on average to be helpful (mean = 75.3mm, S.D. = 2.18mm). Of the 79 matching questionnaires, 67 provided information regarding how the perfusion SPECT influenced their understanding of the patient's disease. 94% (*N* = 63) of clinicians felt that the imaging improved their understanding of the patient's disease, with 5% noting that it either confused (*N* = 2) or did not contribute (*N* = 2) to their understanding.

7.6 Discussion

In this study, we investigated the utility of perfusion SPECT imaging in aiding diagnosis and diagnostic confidence in a large clinical sample of patients with cognitive complaints. We show that perfusion SPECT imaging has good utility to provide added value over standard clinical diagnostic workup alone, demonstrated by a change in diagnosis after imaging in 44% of cases in our sample. Perfusion SPECT was most useful in cases where there was high diagnostic uncertainty, with a large increase in confidence seen when a clinician was uncertain about diagnosis (had less than 50% confidence in their diagnosis before imaging). Clinicians with moderately high pre-imaging confidence in the diagnosis did not change confidence levels, and interestingly, a number of clinicians with the highest confidence levels became less certain about the diagnosis following imaging results. Overall, the majority of clinicians found that the perfusion SPECT imaging contributed to the diagnostic process (96%) and improved their understanding of their patient's disease (87%).

We found over 40% of cases changed diagnosis after imaging in our sample. Previous studies investigating change in diagnosis after diagnostic investigations have shown varying results, with diagnosis changing in 9% to 27% of all cases after FDG PET, amyloid PET and Alzheimer's disease CSF biomarkers (192,306,307,310). Where it was recorded, mean diagnostic

confidence before investigation in these studies was however higher than in our sample, and change in diagnosis increased to 38% in diagnostic 'dilemmas' (307) and to 45% in individuals where clinicians were less than 60% confident before imaging (192). Our results are in keeping with this literature, however direct comparison of confidence change in separate studies is difficult due to differences in investigation type, clinician experience, reason for imaging and patient selection.

We also found a large discrepancy between imaging result and clinical diagnosis in our sample. Similarly poor correspondence has been found when clinical diagnosis was compared to FDG PET disease patterns (192). The discordance seen could be due to a number of factors. Our sample was relatively young (average age 67 years old), with younger dementia patients known to show atypical presentations that can overlap diagnostic subtype categories and take longer to diagnose than late onset dementia (311,312). Significant diagnostic uncertainty was also present in the sample despite prior clinical investigations and although clinicians involved were all at associate specialist or consultant level, clinician skill and experience in dementia diagnosis will vary. Further conflict between imaging and clinical diagnosis could have arisen due to questionnaire formatting. Difficulty with the use of the term mild cognitive impairment was observed in the study, with some clinicians using the term as an intermediate for individuals with cognitive symptoms but no evidence of a dementia subtype (a functional concept), while others used it as a pre-cursor to Alzheimer's disease (a neuropathological concept). Division of MCI into stable and progressive subtypes in the questionnaire may have provided further clarification for these individuals.

Difficulty in diagnosis was most notable in suspected frontotemporal dementia, where a diagnosis of FTD remained after imaging in only 40% of individuals. Difficulty in making an FTD diagnosis is well described in the literature, with clinical criteria for distinguishing FTD from AD previously criticised (313), although these criteria have since been revised (47). Additionally, individuals with AD can present with psychiatric and behavioural symptoms (293–295) and do not always present with memory complaints (110,179,311), making AD difficult to distinguish from FTD in the early stages of the disease when frontal or temporal predominant atrophy may not be present (314). Phenocopy syndrome of bvFTD, where patients present with

behavioural and cognitive symptoms that fulfil the 'possible' diagnosis criteria however show no functional decline, can further confuse diagnosis (315,316).

Although there were differences between pre-imaging diagnosis and SPECT results, clinicians tended to align their diagnosis with the imaging. When perfusion SPECT disagreed with original diagnosis, diagnosis changed in over two thirds of individuals. When it agreed, diagnosis remained stable in all but one case.

We found that imaging was most effective in aiding clinician diagnostic confidence when there was substantial uncertainty (i.e. less than 50% prior confidence). Similar findings have been shown with FDG and amyloid PET imaging (192), suggesting that functional imaging techniques can provide information beyond standard diagnostic workup that can aid diagnosis in particularly difficult cases. Additionally, we found that imaging destabilised the confidence of some clinicians who were very confident pre-imaging, with an average decrease in confidence in this group. Further investigation of clinicians with high pre-imaging confidence found that when imaging confirmed pre-imaging diagnosis, confidence remained stable or was further increased and diagnosis remained. In two cases imaging agreed but was not diagnostic, leading to a small decrease in confidence but no change in diagnosis. When imaging disagreed, confidence decreased in all cases, with diagnosis changed to reflect imaging in two cases and changed from a diagnosis of MCI to AD with a dramatic decrease in confidence in one case (directly conflicting with the imaging result). In the remaining case where MCI was given as the pre-imaging diagnosis, imaging was reported as normal and MCI diagnosis remained but confidence decreased. The decrease in confidence for those with high pre-imaging confidence may not be a negative aspect of the imaging, but an effect of making the clinician reassess their diagnosis when their prior diagnostic expectation was not upheld by the imaging.

Unfortunately, without histology or long-term follow up we were unable to verify the diagnosis of those individuals where clinical diagnosis and imaging conflicted. This study was not however designed to validate the accuracy of perfusion SPECT reporting but to investigate the influence of perfusion SPECT reports with modern methods of analysis on clinician confidence and diagnosis. Previous Chapters presented in this thesis show that imaging patterns on HMPAO SPECT are reliable in dementia diagnosis and relate closely

to DLB and AD pathology as represented on DaTSCAN imaging and CSF biomarkers respectively (Chapters 3 and 4). Additionally, abnormality on HMPAO SPECT is an accurate indicator of future decline showing a strong relationship to neurodegeneration (Chapter 5).

Prior studies investigating the ability of perfusion SPECT imaging to directly influence diagnostic confidence in clinical situations are sparse. A study from 1995 by Van Gool et al assessing usefulness of perfusion SPECT found that clinicians rated that SPECT imaging influenced their level of confidence in only 8% of subjects (57). Our study results directly conflict with these findings, with perfusion SPECT imaging significantly improving diagnostic confidence on average by over 25% in those with the largest diagnostic doubt. The increase in confidence change and utility seen in comparison to the Van Gool (1995) study is likely due to differing methods related to technological advancements made since the study was published. The present study used a modern scanner and individual against control group semi-quantitative analysis, compared to the now outdated scanner and visual and manual region of interest analysis seen in the Van Gool (1995) study. Additionally, knowledge on functional imaging patterns and dementia subtypes have grown considerably over the past twenty years since the Van Gool (1995) study was published, with extensive literature aiding image reporting accuracy.

There are limitations to this study. As information on confidence was collected during normal clinical care across multiple sites, gold standard pathological testing and long-term validation of patient diagnosis was unavailable. We were also unable to determine if other tests were completed between the time of pre-imaging and post-imaging questionnaire completion. Although this is a single centre test, patients were referred from a wide geographical area (Hampshire, Dorset, Isle of Wight and southern Wiltshire), therefore information was gained from a large number of clinicians, with different levels of experience, across different disciplines providing a representative sample to determine clinician opinion. Data on exact number of years of clinician experience was not however collected in the study. Finally, although SPM has been shown to be a useful method of analysis to aid diagnosis by identifying dementia disease patterns (see Chapter 3 and Chapter 4), in clinical practice these patterns still require an experienced clinician to report on the images. Imaging accuracy in diagnosis is therefore limited by current dementia pattern

knowledge in both the current literature and of the reporting clinician. In this study, a single image reporter was used for all image interpretations as per the current clinical process at UHS. A consensus diagnosis of multiple clinicians on each image would provide further reliability to results, however this was not feasible due to the clinical nature of the study. There may also be a 'respected reporting clinician' influence, with clinician diagnostic confidence being further boosted by their respect for the reporting clinician.

In conclusion, perfusion SPECT imaging shows additional value beyond standard diagnostic workup and improves clinician confidence in diagnosis. Perfusion SPECT can provide a diagnosis where none is available, aid specification of individual dementia subtype where dementia is suspected and confirm dementia diagnostic subtype for individuals in whom diagnosis is already certain. Perfusion SPECT is especially useful in cases where clinicians have lower diagnostic confidence, however it can also aid clinicians with the highest diagnostic confidence by both confirming diagnosis and prompting a reassessment of diagnosis when prior expectation is not upheld by the imaging. In our sample, the vast majority of clinicians found that the imaging contributed towards the diagnostic process and the clinicians understanding of the patient's disease, even when the imaging conflicted with their original diagnosis. Results from this study validate the use of HMPAO SPECT to aid dementia diagnosis and show that perfusion SPECT imaging with modern methods of analysis is valued by clinicians and contributes to diagnostic decision making.

Chapter 8: Conclusions

The rapid advancement of imaging techniques in recent years has brought into question the continued use of rCBF SPECT to aid dementia diagnosis and prognosis, with older qualitative visual methods of analysis having limited accuracy and reliability for detecting dementia. Application of newer methods of quantitative and semi-quantitative analysis, such as statistical parametric mapping, have been shown to improve dementia diagnostic accuracy of rCBF SPECT, however these have required clinical validation, with a lack of translational studies directly applicable to clinical scenarios.

This thesis outlines five studies that used unselected clinical samples with focus on the utility of perfusion SPECT imaging using quantitative methods of analysis for dementia diagnosis, prognosis and prediction of care needs in the individual. In addition, the value of perfusion SPECT using updated methods to aid clinician diagnostic confidence and influence diagnosis in clinical practice was determined.

8.1 Validation of rCBF SPECT in dementia

8.1.1 Diagnosis and prognosis

Information on perfusion SPECT imaging patterns which reliably indicate dementia subtype in unselected clinical samples has particular value for the clinician, especially in cases where multiple differential diagnoses exist. In Chapters 3 and 4, we confirmed the ability of quantitatively analysed perfusion SPECT imaging to accurately identify DLB and AD individuals as determined by independent antemortem gold standard biomarkers (DaTSCAN imaging and CSF protein biomarkers) and we defined patterns that can be used to aid dementia diagnosis in the individual for these subtypes.

The combination of occipital lobe perfusion deficits with posterior cingulate gyrus preservation was found to be a specific biomarker for DLB in a sample of individuals with both rCBF SPECT and DaTSCAN dopamine imaging completed as part of clinical care. These *a priori* regions of interest showed very high specificity and good positive likelihood ratio (11.1) when used in combination

for detection of abnormal DaTSCAN imaging and therefore DLB individuals. Sensitivity and NPV was low, suggesting that the absence of the sign cannot be used to rule out DLB.

In a sample of individuals with CSF biomarkers (amyloid-beta, total tau and phosphorylated tau), perfusion SPECT deficits in the inferior parietal lobe, particularly of the angular gyrus, and precuneus, were highly predictive for those with abnormal CSF biomarkers typical for AD. Additionally, specific regions on perfusion SPECT imaging could be used to differentiate patients with low AD likelihood from those with high AD likelihood based on combined CSF biomarkers, with correct classification of 50 out of 68 patients using a leave-one-out discriminant analysis. Analysis of individual SPECT perfusion maps when compared to controls identified common regional abnormalities in each CSF biomarker group, with the angular gyrus showing the most consistent regional abnormality in AD individuals.

The longitudinal analysis presented in Chapter 5 highlights the ability of perfusion SPECT to identify individuals who are most likely to decline. In our sample, individuals with abnormal imaging showed faster rates of decline and a higher probability of reaching the predetermined functional decline outcome on the CDR than those with normal imaging, validating the ability of perfusion abnormality on quantitative SPECT to detect neurodegeneration. We also postulate that different perfusion SPECT imaging patterns may have different decline rates, with patients having predominantly temporal abnormality showing a slower decline rate than those with frontal, parietal and occipital abnormality. This offers the prospect of improved prediction of prognosis at individual level for people with early dementia using rCBF SPECT.

Investigation of perfusion SPECT accuracy for prediction of dementia in this work show the true sensitivity and specificity of rCBF patterns for prediction of DaTSCAN imaging, combined CSF biomarkers and longitudinal decline status, when used in a mixed clinical sample. Sensitivity and specificity rates obtained in Chapters 3, 4 and 5 are summarised in Table 8-1 and are similar to meta-analysis results from rCBF studies of single dementia subtype against subtype, and subtype versus non-dementia comparisons (75,76,173). Importantly, results show that specific patterns of regional abnormality on perfusion SPECT have high specificity and positive predictive values for DaTSCAN and CSF biomarker status (specificity 98% and 89%, PPV 92% and 91% respectively), and

the presence of any rCBF abnormality has good sensitivity and negative predictive values for prediction of functional decline (sensitivity 81%, NPV 85%).

Although specificity was relatively low for prediction of decline on CDR, our follow up time for the study was limited (18 months). During a longer follow-up period we would expect more individuals with abnormal imaging to reach the decline endpoint, as suggested by prior longitudinal investigation of CDR status (317). Longer-term follow up would therefore be expected to increase prediction accuracy and distinction between survival curves for individuals with normal and abnormal imaging. Longer follow up would also provide further assurance that those who met the decline outcome in our study have irreversible decline and do not include individuals with cognitive impairment that revert back to cognitively normal ('reverters') (318,319). 'Reverters' were not considered in the analysis presented, however the use of caregiver informers who see the patient regularly between follow up points, rather than direct patient cognitive assessments for monitoring of decline, should limit any potential effect that may arise from short term transient fluctuations in cognition.

Knowledge of regional pattern sensitivity and specificity in detecting dementia subtypes in people with cognitive complaints suggestive of underlying dementia is essential for clinicians reporting scans to weight their clinical judgement accordingly. Rates of sensitivity and specificity throughout this body of work are however dependent on selected imaging and biomarker cut-points, with an arbitrary 2 standard deviation away from control mean cut-point chosen to define perfusion SPECT imaging abnormality. Due to the continuous nature of biomarker data, results close to cut-points should be interpreted with care. Optimisation of cut-points may provide further sensitivity and specificity gain; however standardisation of methods across sites is needed to ensure cut-points are reliable in different centres. Despite a recent drive for cut-point and methodological standardisation for CSF AD biomarkers, amyloid PET, FDG PET and MRI cortical thickness analysis (320-323), there have been no large multi-site studies for standardisation of methods or to derive standard cut-points of abnormality for HMPAO SPECT. With the utility of HMPAO SPECT using quantitative methods of analysis established in this body of work, standardisation of methodology and cut-points is required to ensure comparable accuracy between sites.

Overall, the cross-sectional and longitudinal validation of rCBF SPECT with newer analysis methods presented here adds to current knowledge by confirming the technology's ability to detect dementia in clinical samples where pre-imaging diagnosis is unknown. Knowledge of validated patterns of abnormality in the individual can be used to aid image reporting and suggest that deficits on HMPAO SPECT are sufficient for diagnosis in some individuals without the need for further investigation. Additionally, individuals with normal perfusion on SPECT rarely show functional decline indicative of dementia, and those with poor rCBF at baseline are at high risk of decline. Different patterns on perfusion SPECT may also be able to provide further prognostic information on the individual for the clinician and with further elucidation of these patterns, could provide added discrimination of those at risk of poor outcome.

8.1.2 Identification of care needs

While perfusion SPECT imaging offers diagnostic and prognostic value in dementia care, there is further information that can be gained from rCBF scans that is rarely utilised in clinical practice. Typical phenotypes for each dementia subtype are well-known and outlined in multiple dementia diagnostic guidelines, and there are a plethora of online platforms aimed to inform and prepare the caregiver for symptoms that the patient may experience throughout the lifetime of the disease. Manifest symptoms are typically directly related to the location of underlying cerebral pathology, and therefore imaging pathology can flag potential symptom presentation (49), offering a unique opportunity to identify care needs.

Our investigation of the relationship between patient regional perfusion on HMPAO SPECT and caregiver burden in Chapter 6 showed that individuals with frontal or right sided pathology, regardless of diagnosis, were more likely to place high burden on their caregivers than those with left parietal or no pathology. This relationship between caregiver burden as assessed by the Zarit burden inventory questionnaire and patient imaging remained after patient dementia severity was considered. The work presented suggests that the location of imaging deficits could be further utilised by clinicians, in conjunction with typical subtype phenotype information to best identify individuals who may present with increased care needs. This information can then be used to inform and prepare the patient caregiver of the potential for

challenging symptoms, and help clinical services anticipate future care or caregiver support needs.

We note the age of our sample in this analysis is relatively young (mean age 69 years old, S.D. 8.34), with some individuals having onset of symptoms before the age of 65 years old. Caregivers of younger dementia sufferers can experience particular psychological distress related to their phase in life and have been shown to have higher levels of burden than those caring for older individuals (324,325). Furthermore, caregiver burden levels were obtained around the time of patient imaging, where diagnostic uncertainty may have influenced anxiety and perceived difficulties in caregiving (292,324,326–328). Follow up comparisons of imaging and caregiver burden levels after diagnosis would provide evidence for long term prognostic value of imaging for care needs, however is unlikely to be practical for most individuals. The proportions of each dementia subtype and non-dementia pathology in the sample was also unknown, having potential to influence the resulting imaging patterns and their relationship to behavioural symptoms and burden.

8.1.3 Diagnostic confidence and change in diagnosis

Despite the ubiquity of rCBF SPECT imaging in dementia in the UK, literature on the influence of the technology on the diagnostic process is extremely limited. There has been a solitary study, completed over 20 years ago, on how perfusion SPECT influences diagnosis and diagnostic confidence (57). As diagnostic confidence is ultimately what determines patient management, updated knowledge on the effect of current perfusion SPECT with semi-quantitative methods of image reporting on confidence and diagnosis is certainly beneficial. We show in Chapter 7 that perfusion SPECT imaging with newer analysis methods gives results perceived to be valuable by clinicians in diagnosis in a sample where there was significant prior diagnostic uncertainty. Perfusion SPECT altered diagnosis in over 40% of cases and in clinicians who were less than 50% confident in their diagnosis before imaging, diagnostic confidence significantly increased after imaging on average by 26%. Although there was not a significant overall change in diagnostic confidence for those with a pre-imaging confidence greater than 50%, perfusion SPECT imaging did alter confidence in individual cases, and 96% of all clinicians felt that the imaging contributed to the diagnostic process. Just as importantly, where

clinician prior confidence was high, SPECT imaging had the potential to reduce inappropriate confidence where the prior clinical judgement was quite likely to be incorrect.

Results of change in diagnostic confidence and diagnosis found are similar to that seen in FDG PET, amyloid PET, AD CSF biomarkers and MRI automated hippocampal volumetry despite apparent differences in accuracy between these techniques stated in the literature (192,307,308). This information can be used to further reinforce rCBF SPECT use with newer analysis methods for dementia diagnostics, but results require validation in other centres. As stated in Chapter 7, effectiveness of perfusion SPECT with semi-quantitative analysis depends on both the state of current literature and reporting clinician knowledge on imaging patterns. The vast majority of research on perfusion SPECT patterns for dementia focus on the AD subtype, and further validation of rCBF SPECT imaging patterns in FTD (both bvFTD and PPA subtypes), VaD and other less common dementia subtypes (such as CDB or PSP) is still needed. It is also essential that reporting clinicians in perfusion SPECT imaging test centres keep up to date with current knowledge of imaging biomarker patterns, such as those defined in Chapters 3 to 6. The production of visual scales that identify common perfusion SPECT imaging patterns on SPM and their associated dementia subtype may be beneficial to ensure that reporting standards for dementia diagnosis are consistent between sites.

The high rate of change in diagnosis and influence on diagnostic confidence in this study illustrates the impact of this biomarker in routine clinical practice. The results suggest that clinicians should utilise perfusion SPECT imaging to aid diagnosis, when available, in cases where there is significant diagnostic uncertainty. Investigation of the cost-effectiveness of perfusion SPECT imaging and change in management or treatment after imaging would provide further real-world evidence on its continued use in dementia diagnosis and prognosis, particularly when compared to its imaging counterparts (FDG PET and MRI / CT imaging).

Table 8-1 Accuracy rates of rCBF to predict DaTSCAN, CSF and decline status

Showing accuracy, sensitivity, specificity, PPV and NPV rates obtained throughout this body of work. Further detail is provided in Chapters 3, 4 and 5. * Designates higher perfusion in abnormal or high risk groups.

	Accuracy	Sensitivity	Specificity	PPV	NPV	Regions used
DaTSCAN abnormality	61%	22%	98%	92%	56%	Posterior cingulate*, Middle and lateral occipital
CSF AD any abnormality	86%	84%	89%	91%	79%	Left angular gyrus, Left superior occipital, right medial temporal, posterior cingulate
CSF AD low risk vs high risk	74%	67%	77%	56%	83%	Left angular gyrus, right middle temporal pole*
Longitudinal decline	62%	81%	52%	45%	85%	Any regional abnormality

8.2 Considerations for the future

While there is strong evidence for the continued use of perfusion SPECT imaging in dementia diagnosis and prognosis with application of newer methods of analysis, a number of outstanding questions remain.

- In a large clinical sample of individuals with cognitive complaints, are decline rates different for distinct patterns on rCBF SPECT, and can knowledge of imaging deficits provide additional prediction for future decline over dementia subtype diagnosis?
- With the knowledge that specific imaging patterns on rCBF SPECT are valid for dementia subtype diagnosis, could the use of automated machine learning methods of analysis be used to good effect in heterogeneous clinical samples, and if so, would they be accepted by clinicians?
- What is the optimal time for the use of perfusion SPECT imaging in the patient diagnostic pathway that provides the most value in clinical practice?
- Is wide-scale implementation of semi-quantitative and automated analysis methods for HMPAO SPECT sites currently using visual imaging analysis feasible, and would its application provide further diagnostic confidence and accuracy across multiple sites?

Results presented from the longitudinal BrainID study in Chapter 5 suggest that different rCBF patterns may show different decline rates, a finding that could be used to further aid prognosis in clinical scenarios. Due to limited group sizes, these results need validation in a larger sample study with longer follow-up. Longitudinal verification of diagnoses for individuals would also be useful, allowing investigation of whether imaging patterns can provide added prognostic value over dementia subtype diagnosis.

With the advent of machine learning techniques such as neural networks and support vector machines, it is now possible to automate detection of abnormal imaging patterns indicative of dementia subtype. Previous investigation of these techniques using research samples in perfusion SPECT imaging has been completed and show good prediction accuracy for AD (44,45), however the

technique has not been tested in clinical samples. It is unknown whether machine learning methods of analysis could provide a reliable method of dementia subtype prediction in heterogeneous cognitive impairment samples. Patterns elucidated in Chapters 3 and 4 for DLB or AD prediction in this thesis could be used to validate machine learning derived patterns, or the samples used to train the learning models to detect individuals with high likelihood of abnormal biomarkers of DaTSCAN or AD CSF. Automated detection of regional abnormality, such as the posterior cingulate island sign as defined in Chapter 3, could be used to alert clinicians to high risk individuals to further aid diagnosis. Additionally, machine learning techniques are flexible enough to allow a multimodal approach to data analysis. Perfusion SPECT biomarkers could be combined with other available clinical data from cognitive or biomarker tests to further improve disease detection. The combination of perfusion SPECT and FDG PET with MRI and CSF biomarkers has been shown to improve detection accuracy over individual biomarkers when used separately (165,177,329–331).

We have shown in this body of work that perfusion SPECT imaging provides good accuracy for dementia detection and influences clinician diagnostic confidence and diagnosis, however it would be beneficial to further explore the added value and diagnostic impact of rCBF SPECT over specific clinical assessments and structural imaging (CT and/or MRI). Investigation of when perfusion SPECT imaging provides the most value and for which patients, could help improve the patient pathway, reducing time to diagnosis and implementation of care.

Although we did not directly compare the use of visual and quantitative analyses of HMPAO SPECT, this has previously been completed (22,35–37), with our results adding further weight to the argument for the use of quantitative analysis of rCBF SPECT in clinical practice. With SPM reported patterns providing clinical utility to the reporter, this thesis provides evidence for the uptake of this method in centres which currently use visual reporting methods. The feasibility of the uptake of the analysis is however unknown, and a study that explored the potential benefits of and barriers to dissemination of the technique would be informative.

In conclusion, regional abnormality on perfusion SPECT imaging using newer methods of analysis is useful for both identification of dementia subtype and

future decline and provides the clinician with information on potential care needs of the patient and their caregiver. rCBF SPECT also influences diagnosis and diagnostic confidence, and clinicians value the technique in the diagnostic process. This body of work provides translational evidence on the clinical utility of perfusion SPECT imaging in real-world clinical practice and supports the use of the technique using modern quantitative and semi-quantitative methods of analysis in dementia.

Appendices

Appendix A

- The Retrospective brain imaging in dementia study (RetroBraIID)
    - Protocol

Appendix B

- The Brain imaging in dementia study (BraIID)
    - Protocol
    - Patient, caregiver and consultee information sheets
    - Patient, caregiver and consultee consent forms
    - General practitioner (GP) letter
    - Demographics questionnaire

Appendix C

- Clinician attitudes to diagnostic investigations (CADI) study
    - Questionnaires

Appendix D

- Statistical parametric mapping settings

Appendix A

A.1 RetroBrainID protocol

The Retrospective Brain Imaging in Dementia study

Background and objectives

It is estimated that there are 800,000 people with dementia in the UK currently, costing £23bn a year, a figure that is projected to rise with the ageing population. Early, accurate diagnosis is a core part of the National Dementia Strategy, and is frequently identified as a key need for patients and carers (National Dementia Strategy, 2009; World Alzheimer report, 2011). Clinically, diagnosis and prognosis in dementia is often difficult, with symptoms, functional difficulties and imaging pathology commonly varying between patients. Delayed diagnosis can lead to a delay in assignment of care resources, as well as add to anxiety of families already in distress. Early diagnosis and identification of individuals at highest risk of decline is essential for signposting patients to appropriate care resources.

Currently, diagnosis and prognosis (outcome of a disease) is aided by cognitive screening assessments, brain imaging (functional and structural) and cerebrospinal fluid biomarkers (CSF). (99m)Tc-HMPAO single-photon emission computed tomography (SPECT) imaging is commonly used in preference to other diagnostic brain imaging modalities, however reporting standards vary widely between sites with few using objective measures to help adjudicate diagnostic decision-making. There is also poor data on the long-term outcome of SPECT imaging patterns and how these imaging patterns in the individual can help inform prognosis, rate of functional decline or the need for additional care.

Definition of specific patterns which reliably predict disease and decline as well as optimisation of clinical assessment tests to predict imaging abnormality will assist in earlier diagnosis and could reduce the extent of diagnostic testing required for confident diagnosis in an individual.

This study aims to maximise the use of imaging data previously acquired during routine clinical care for those with dementia to improve diagnostic and prognostic accuracy in dementia clinical practice. The study will use pseudonymised retrospective data collection and analysis to study the value of imaging, fluid biomarkers and neuropsychological tests in a cognitively impaired group.

Study Design

An observational, retrospective, single-centre study using pseudonymised data only.

Study Population

This study will obtain retrospective clinical data from patients with cognitive complaints who have been seen in University Hospital NHS Foundation Trust (UHS). Patients will fall into two main groups:

1. Patients who have had nuclear medicine brain imaging for cognitive complaints (HMPAO or DaTSCAN Single photon emission computed tomography) or Cerebrospinal fluid dementia biomarkers completed;
2. Patients who have been seen in the cognitive clinic for cognitive assessments, behavioural and functional tests

Patients will be between the ages of 18 and 100 years old. We will collect as many data records as possible for this study, commencing from the year 1997. We predict this to be around 2500 data records.

Data collection

All data used in the study will be pseudonymised to researchers outside the patient's normal clinical team. Clinical data will be obtained from the UHS nuclear medicine department clinical database, clinical picture archiving and communication system and the cognitive service clinical records. Data will be obtained and pseudonymised by a member of the clinical team (CK, AP), who will remove all patient identifiable data. Only the pseudonymisation code and HMPAO-SPECT study number (number only code not related to patient hospital number or date of birth) will be available to the researchers. This will allow data across modalities to be linked.

This is a retrospective review of existing data, and we will not be making contact with any individuals during this study. This study will not use patient identifiable data and all images used will be pseudonymised.

Data Management and Confidentiality

Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing all imaging and clinical data. All data collected will be kept on password protected hospital computers on a shared drive and will be kept on secure premises. Patient identifiable data will not be available to researchers outside the main clinical team. Data collected will be stored for a maximum of 20 years.

Ethical Considerations

This study will be using pseudonymised data only. The study will not involve participants, and therefore involves no experimental treatment and no invasive procedures.

Statistical Analysis

This is a retrospective observational data only study of the value of imaging, fluid biomarkers and neuropsychological tests in a cognitively impaired group. In view of this there will be co-primary outcome measures for this study.

The main outcome measures are:

1. Rate of decline in cognitive, functional and behavioural scores
2. Correlation of imaging patterns with cerebrospinal fluid biomarkers
3. Correlation with clinical outcome (where known)

We will analyse the data using observational analysis techniques. These will include:

1. Simple correlation and linear and logistic regression of cognitive functional and neuro-behavioural scores on imaging and fluid biomarkers

2. Time series modelling on longitudinal decline towards a biomarker defined end point (imaging or Cerebrospinal fluid) or institutionalisation due to cognitive factors or death
3. Cross-correlation of regional and global imaging across modalities

Study contacts

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Appendix B

B.1 BraIID protocol

PROTOCOL

The Brain Imaging in Dementia Study (BraIID): an observational longitudinal study to optimise image reporting in dementia clinical practice.

Version 1.8, 21st December 2015

Appendix B

Study Sponsor

University Hospital Southampton NHS Foundation Trust

Study Centres

University Hospital Southampton NHS Foundation Trust (UHS)
University Hospital Bristol NHS Foundation Trust (UHB)

Background

Diagnosis of dementia and prediction of decline in individuals with cognitive complaints is often difficult, as symptoms, cognitive deficits and imaging pathology commonly vary between patients. Early diagnosis and identification of individuals at highest risk of decline is essential for signposting patients to appropriate care resources.

Diagnosis is currently aided by cognitive screening assessments, brain imaging (functional and structural) and various biomarkers of neurodegenerative pathology (NCCMH, 2007). In the UK (99m)Tc-HMPAO single-photon emission computed tomography (SPECT) imaging is commonly used in preference to other diagnostic brain imaging modalities for diagnosis, however reporting standards vary widely between sites with infrequent use of sensitive objective measures. There is also little data available on the prognostic utility of commonly identified patterns seen on SPECT image analysis.

This study aims to maximise the use of imaging data acquired in the course of providing routine clinical care and combine this with long-term follow up information on individuals with cognitive complaints in order to improve diagnostic and prognostic accuracy for the individual. The study and outcome measures are pragmatic, and reflect the nature of clinical practice in dementia.

Objectives

The project aims to:

1. Prospectively validate diagnostic and prognostic utility of imaging patterns seen on HMPAO-SPECT in individuals with cognitive difficulties and dementia.
2. Evaluate the effectiveness of HMPAO-SPECT in producing a change in clinician diagnostic certainty in dementia.

Outcomes from this project include improved image reporting standards; improved diagnostic certainty; improved prognostic accuracy and identification of individuals at the highest risk of decline.

Appendix B

Methods

Study Design

Observational, prospective, multi-centre data study without experimental treatment.

Study Period

This study will have an enrolment period of 2 years. Follow up data will be collected for up to 2 years post recruitment closure.

Study Population

Inclusion criteria

Subjects will be those over the age of 18 with cognitive difficulties of any kind and who are scheduled to have a HMPAO-SPECT scan. This study aims to recruit all subjects who are eligible and willing to participate across all centres. We will also recruit companions of those who are scheduled to have an HMPAO-SPECT scan.

Sample size

This study will recruit a minimum of 500 patient participants plus approximately 500 companions over 2 years.

Study enrolment

Potential participant identification (screening)

Participants will be identified through the nuclear medicine department referral lists at participating institutions. All patients over 18 years of age who are due to have a HMPAO-SPECT scan will be sent the study patient information sheet (PIS) and companion information sheet with the nuclear medicine appointment letter in the post. It will be made clear that consent for the study is not a prerequisite for the investigation. As the appointment letters are sent to the patient well in advance of the scan date, patients will usually receive the PIS at least a week before the date of their scan (and study consent). Patients will always receive the PIS at least 24 hours prior to consent as per common Good Clinical Practice standards.

Participant informed consent

Consent will take place on the date of the HMPAO-SPECT scan for the majority of patients and companions, however if appropriate consent can be completed before or after the date of the SPECT scan. Consent will always be completed within 3 months of the scan date. The study will be explained to the patient and companion, and any questions or worries will be answered sufficiently by the study site investigator. During the discussion, the study site investigator will assess the patient for mental capacity to give informed consent for the study. If the patient is assessed to have capacity and they are happy to participate, consent will be taken on the BRAIID study consent form. Where a potential participant is assessed not to

Data collection

Imaging data

Image acquisition

The project will obtain HMPAO-SPECT images for all participants. Previous and future clinical scans of participants, including DaTscan ([123 I] SPECT), Magnetic Resonance Imaging (MRI), Positron emission tomography (PET) and Computed Tomography (CT) images will be collected. Images will be stored as per the current site medical physics protocol.

Image processing

Upon HMPAO-SPECT image acquisition, images will be reconstructed using a filtered back projection (fbp) or ordered subset expectation maximisation (OSEM) method. After this, images will be spatially normalised, smoothed and cerebellar normalised using statistical parametric mapping software (SPM8). Regions of interest (ROIs) will be created using the automatic anatomical labelling (aal) atlas. Measures of perfusion will be calculated from the HMPAO-SPECT images for the ROIs using SPM8. These ROIs will then be combined to create 6 disease 'signature' patterns based on previous literature and clinical knowledge.

Classification of images

Measures of perfusion will be used to classify images into abnormal / normal when compared to controls for both global and ROI pattern analysis. Images will also be classified as abnormal / normal based on visual interpretation both before SPM8 processing and after SPM8 analysis. This will allow for direct comparison between the diagnostic accuracy of quantitative HMPAO-SPECT analysis and subjective visual HMPAO-SPECT.

Overall, processed images will be grouped into:

a) Expert visual interpretation global abnormal / normal of reconstructed images before SPM8 processing (qualitative)
b) Expert visual interpretation of global abnormal / normal of SPM8 Z-map when compared to controls (semi-quantitative)
c) Global abnormal / normal calculation using perfusion measurements when compared to controls (quantitative)
d) Signature pattern (ROI) abnormal / normal calculation using perfusion measurements when compared to controls (quantitative)

Participant clinical data

Baseline clinical data collection

At the time of consent, patient participants will be asked to complete a short form giving basic demographic information, past medical history and medications taken. If the patient is assessed to lack capacity to consent, their companion will be asked to complete this initial form. The companion participant will be asked to complete the Clinical Dementia Rating sum

of boxes (CDR-SoB) symptom inventory, the Functional Activity Questionnaire (FAQ) functional assessment, Zarit caregiver burden questionnaire (Zarit) and the Cambridge Behavioural Inventory (CBI) behavioural assessment. Participant diagnosis, clinical screening test results, symptom inventories, pathology results (related to the participant's cognitive impairment) and functional information will be obtained from the clinical referral, referrer, General Practitioner (GP) or care centres or electronic health records where available.

Participant follow up

Outcome data on progressive change will be obtained from the patient's companion (if recruited) by telephone or letter. Two weeks after consent, a telephone CDR-SoB will be completed with the companion by a trained member of the research team. After this, the outcome data will be collected from the companion at 6 monthly intervals. Assessments collected will include the CDR-SoB, FAQ, Zarit and CBI scales in addition to information on patient diagnosis, date and date of death (if relevant), critical events and their dates, institutionalisation and implementation of a care package. Other assessments completed by the participant as part of their dementia clinical care may also be collected through the primary care centre or electronic regional health record service.

If the patient participant does not have a companion recruited, information will be collected from the clinical referrer, General Practitioner (GP), primary care centres or electronic regional health record service. Information will not be directly collected from the patient participant after the date of consent.

Patient participants are explicitly asked in the consent form for permission for the study site team to access their medical and social care records, contact their GP and to notify them of other studies that they may wish to participate in. Companion participants are explicitly asked in the consent form for permission for the study site team to contact them via email, post or telephone to collect follow up information on their partner. A maximum of three attempts will be made to contact the participant before they will be assumed to be lost to follow-up.

Clinician diagnostic certainty

The diagnostic certainty of the patient's clinician who referred for the HMPAO-SPECT scan will be obtained. Diagnosis and diagnostic certainty will be collected using two questionnaires that will be sent to the referring clinician before and after the HMPAO-SPECT scan is completed. Consent for clinician participation will be implied by the return of the questionnaires. The questionnaires will obtain information on primary and differential diagnoses, clinician confidence in diagnosis, and how the imaging contributed towards diagnosis, diagnostic confidence and clinician understanding of the patients' disease.

Statistical Analysis

Hypothesis

We hypothesise that regional and global perfusion on HMPAO-SPECT can predict:

1. Neurodegenerative disease
2. Longitudinal status / decline (prognosis)

Outcome Measures

Primary Outcome Measure

A composite outcome measure will be defined using:

- Decline on the Clinical Dementia Rating Scale, defined as a change in the global CDR dementia staging group or a change greater than or equal to 2 points per year on the CDR-SoB
- Institutionalisation in a care facility (due to dementia)
- Death (any cause)

Secondary Outcome Measures

Cross sectional status and longitudinal decline on:

- Addenbrookes cognitive examination (ACE-R or ACE-III)
- Cambridge Behavioural Inventory (CBI)
- Mini-Mental State Examination (MMSE)
- Functional Assessment Questionnaire (FAQ)
- Zarit Caregiver Burden test

A change in clinician diagnostic confidence after HMPAO-SPECT imaging.

Exploratory Outcome measures

Serial decline on cognitive scores collected as part of routine clinical care (Mini-Mental State Examination [MMSE], Addenbrookes cognitive examination [ACE-R or ACE-III] or other)

Primary Analysis

Descriptive statistics

Descriptive statistics will be used to assess:

- Sample demographics
- Individual performance (functional, behavioural, cognitive and carer burden scores) relative to pre-specified SPECT patterns at cross-sectional time-points
- Individual performance decline (functional, behavioural, cognitive and carer burden scores) over time relative to pre-specified SPECT disease signature patterns

Longitudinal prognostic analysis of HMPAO-SPECT

Using survival analysis difference in time to event (primary outcome measure) between the global abnormal / normal and pattern abnormal / normal groups will be evaluated. Survival curves of each HMPAO-SPECT signature pattern will be created and decline rates compared.

HMPAO-SPECT pattern prediction of event

Logistic regression and receiver operator characteristic (ROC) curves will be used to evaluate the value of each pattern in predicting primary outcome event occurrence. ROC curves will be used to calculate optimal classification cut-points for the HMPAO-SPECT global and pattern analysis. Prediction values of patterns will be compared to distinguish which patterns best predict event occurrence.

Diagnostic confidence analysis

Evaluation of change in clinician diagnostic confidence after HMPAO-SPECT imaging will be completed. A 100 point visual analogue scale will be used to measure diagnostic confidence before and after HMPAO SPECT imaging. Clinician diagnostic certainty will be compared to the outcome of the individual to evaluate clinician diagnostic accuracy rates.

Sample size calculations

For all sample size calculations a two-sided significance level of 5% with 80% power to detect a significant difference between groups was used. Taking into account the uncertainty inherent in estimating dropout and precise group proportions, a sample size of 1000 participants (500 patients and 500 carers) was decided upon.

Image pattern analysis

Global pattern analysis

A test of two proportions was completed, suitable for logistic regression analysis and basic survival analysis (Kaplan Meier test). The sample size was calculated to identify a difference in proportions experiencing the outcome between global abnormal and equivocal groups. Using pilot data of 164 patients group sizes were calculated as 1:1 ratio abnormal to equivocal (40% abnormal, 40% equivocal with 20% normal). Estimates of group proportions for achieving the outcome were taken as 50% VS 30% (global abnormal VS equivocal). A 20% lost to follow up per year was assumed based on previous dementia longitudinal studies. A sample size of 402 patient participants was calculated to give appropriate power for assessment of study primary outcomes. This gives a total of 804 participants including companion.

Subtype pattern analysis

A test of two proportions was again used to determine the sample size required to identify a difference in proportions experiencing the outcome between subtype abnormal and equivocal groups. Group sizes were assumed to be 1:8 ratio subtype abnormal to equivocal (assuming 5% subtype abnormal, 40% equivocal). Estimates of group proportions for achieving the outcome were taken as 60% VS 30% (subtype abnormal VS equivocal). A 20% lost to follow up per year was assumed. A sample size of 688 patient participants was calculated, giving a total of 1376 participants including companions.

Clinician diagnostic confidence

The sample was sized based on a paired t-test analysis using a 100 point visual analogue scale (VAS) scale to measure clinician confidence. A minimum important clinical difference in confidence of 20 points change between before and after measurements and a standard deviation of 40 points was assumed. Further design effect adjustment was completed to correct for multiple clinicians and clustering of scores. Assuming 10 clinicians will be used and a response rate of 50%, a sample size of 204 was calculated to give appropriate power.

Study Governance

Data Management and Confidentiality

Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing participant data. All identifiable information collected will be kept on password protected hospital computers on a shared drive and all information will be kept on secure premises. De-identified study data will be uploaded to electronic case report forms (eCRF), stored on secure SSAE 16 certified data centres which are compliant with regulatory and security requirements (21 CFR Part 11 and HIPAA) and with the U.S.-EU Safe Harbour Framework. All data collected will be stored for up to a maximum of 20 years.

There are several provisions in place to maintain integrity, confidentiality, and security of participant information. Data will be shared using a participant identification number (PID) pseudonym with no identifying information. Data will be shared securely using post, secure email or a safe haven fax machine. Authorised researchers and other users will only have access to completely de-identified data.

Mental Capacity Act

Participants lacking capacity to consent

As this study is recruiting subjects with cognitive difficulties and dementia, some subjects may not have the mental capacity to give informed consent. It is however expected that the vast majority of patients will be in the early stages of dementia, and have capacity to consent. To ensure participants in the study cover the whole spectrum of dementia disease, potential participants who are assessed to lack capacity will be recruited to the study.

Assessment of capacity will be undertaken by a trained and qualified study site investigator. They will assess whether the potential participant can:

1. Understand what is required of them for study participation.
2. Retain the information.
3. Use or weigh the information to be able to come to a decision.
4. Communicate their decision to the site investigator.

If the potential participant is assessed to lack capacity to consent, a personal consultee will be identified. The personal consultee will be someone who knows the person lacking capacity in a personal sense. The personal consultee will be able to inform the site investigator about the individual lacking capacity's wishes and feelings in relation to

research. The personal consultee will usually be a spouse, carer, family member or friend of the individual.

The personal consultee will be given a personal consultee information sheet which will outline their role as a consultee and provide them with detailed information about the study requirements. If the personal consultee believes that the individual lacking consent would be happy to participate in the study, they will be asked to complete the record of consultation sheet with the study investigator.

Individuals who lack capacity to consent and do not have a companion present will not be recruited. No information will be collected directly from participants lacking capacity.

Participants with reading and writing difficulties

Some subjects recruited to this study are likely to have difficulties in reading and/or writing (due to apraxia, visuospatial deficits, language deficits or other difficulties linked to their condition). Due to this, we have kept the complexities of the patient information sheets and consent forms to a minimum, without removing essential study and governance information.

Ethical Considerations

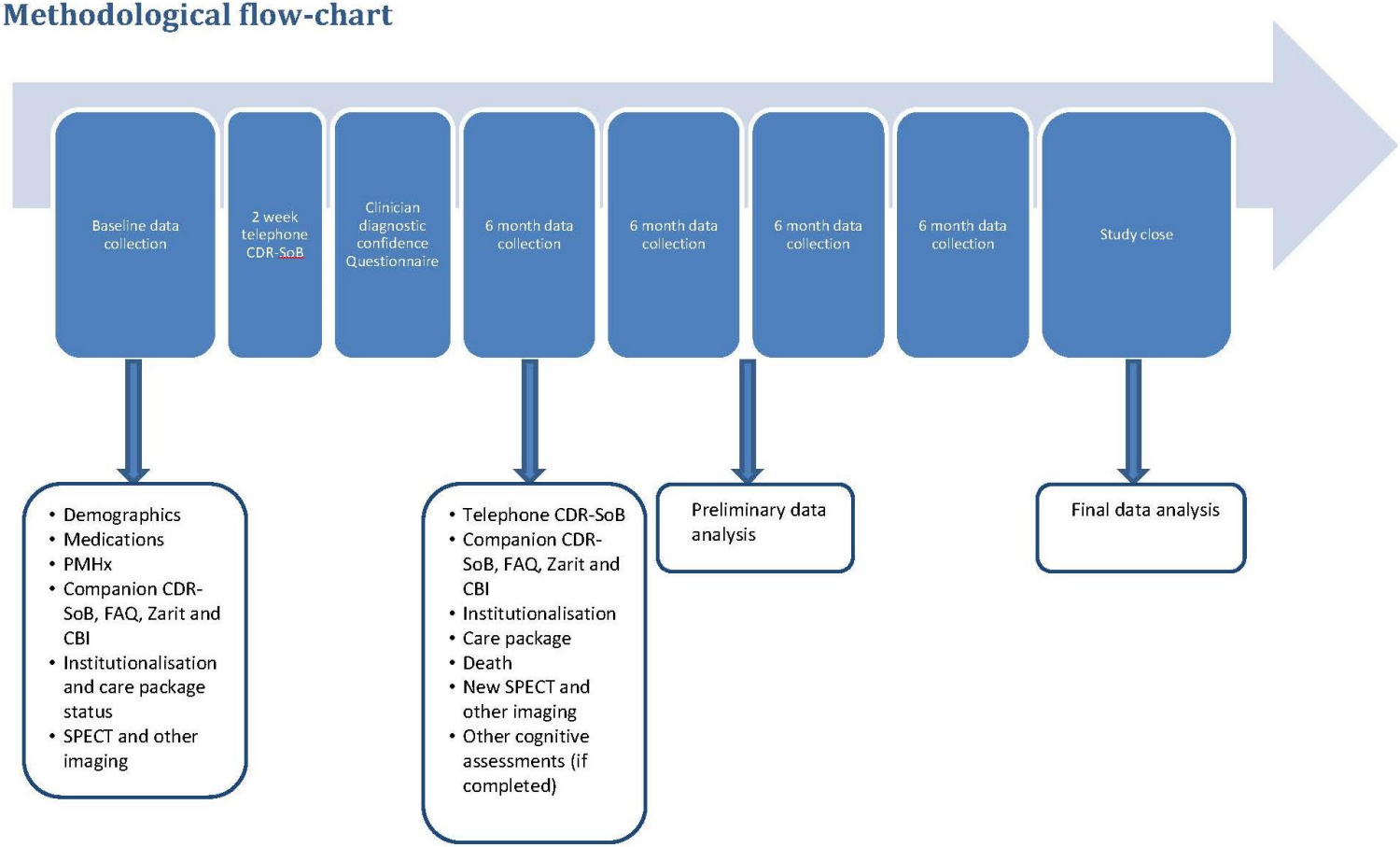
Risks to participants

The study involves no experimental treatment and no invasive procedures are planned. The study protocol does not mandate any diagnostic investigation or any treatment beyond what is undertaken as part of routine clinical care.

As the participant information sheets are sent to potential participants with the clinical appointment letters for the HMPAO-SPECT scan, it is possible that patients may believe that the research is compulsory for their clinical scan to be completed. It will be explicitly mentioned in both the information sheets and the letter inviting potential participants that this research is voluntary and is not part of their normal clinical care, and even if the participant chooses not to participate in the research, their clinical scan will still be completed. In addition, the investigator will explain this to the patient (and companion if present) before research consent is taken.

This research project does not come under Ionising Radiation (Medical Exposure) Regulations 2000 (IRMER), as the HMPAO-SPECT is authorised and undertaken in the course of normal clinical management, not for research purposes; and the decision to authorise the exposures is clearly separated from the decision to include the participant in the research and is not decided in advance by the research protocol. As such, this project does not require Administration of Radioactive Substances Advisory Committee (ARSAC) approval.

Methodological flow-chart



Appendix B

Patient flow-chart (example)

Study contacts

Chief Investigator –	Dr Christopher Kipps, Consultant Neurologist
Principal Investigator UHS –	Angus Prosser, NIHR PhD student and research assistant
Principal Investigator UHB –	Dr Robin Holmes, NIHR/HEE Post Doctoral Research Fellow
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B.2 BraIID participant information sheets and letters

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RESEARCH
Brain imaging in dementia study (BRAIID)

Dear

The hospital is currently running an observational research study looking at people who are having thinking difficulties of any kind and are scheduled to have a SPECT brain scan to look at the activity of their brain. As you are scheduled to have a scan we thought you may be interested in taking part.

Please find enclosed the participant information sheet which gives detailed information about the research study. If you are attending your scan with someone else, there is also a separate companion information sheet included for them.

I will be in contact by phone to find out if you are willing to take part in the study. If you are, you will be asked to come in to the hospital to discuss the study. This can usually be completed on the same day as your scan appointment. If you have any questions about the study or wish to inform me that you are willing to take part, please feel free to get in touch with me at the contact details below.

Please be assured that if you decide you do not wish to participate in the study that this will not affect your care in any way. You do not need to give us a reason if you decide against participating.

Even if you choose not to participate in this study, your scan will still be completed.

With very best wishes,

Angus Prosser

Title: Research Assistant
Telephone: 02381 20 6132
Email: angus.prosser@nhs.net

PARTICIPANT INFORMATION SHEET

Brain imaging in dementia study (BrailD)

Introduction

We would like to invite you to take part in a research study. The aim of this study is to improve our use of brain scans in clinical diagnosis and prognosis (a prediction of the course of a disease) in those with cognitive impairment and dementia.

The study is organised by Dr Christopher Kipps (Consultant Neurologist) and Angus Prosser (PhD student and Research Assistant) at University Hospital Southampton NHS Foundation Trust. The study has been reviewed and approved by the South Central Hampshire A Research Ethics Committee.

Why have I been asked to take part?

You have been asked to take part because you are currently having some difficulties with your thinking and you have been scheduled to have a scan to look at the activity of your brain (a SPECT or PET scan). Every patient who is booked in to have a SPECT or PET scan will be asked if they would like to take part in the study.

What does the study involve?

- We will contact your GP, the physician in charge of your care and/or your companion (if recruited) for some information about you throughout the study.
- We will access and store data from your health care records, including electronic records such as the Hampshire Health Record service. Data collected will include information such as relevant past and current medical history, medications taken, cognitive assessments completed and your brain scans.
- We will continue to collect information from your carer / companion, GP and/or your health records even if you lose the ability to make a decision (capacity) during the study.
- We may also contact you to inform you of upcoming studies for which you may be eligible and that you may be interested in participating in.

If you would like to take part in the study, you will be asked to sign a consent form to show that you have agreed to take part. You will also be asked to fill in a short 5-minute questionnaire with your companion (if recruited) to collect some simple background information about you such as age, current medications, past and current relevant medical problems, education and family history.

The study will not affect or change any of your normal clinical care. Even if you choose not to participate in the study, your scan will still be completed.

Appendix B

Do I have to take part?

It is up to you whether or not to take part in this study. If you do decide to take part, you can withdraw at any time without giving a reason. If you decide you do not want to take part, it will not affect the standard of care you receive in any way and you will still receive your scan.

Will my information be kept confidential?

All information that is collected about you will be kept in the strictest confidence in accordance with the Data Protection Act 1998. All information collected as part of this study will be stored under a code rather than your name and will have your address removed. The information will be kept on password protected computers and all information will be kept on secure premises. All data collected will be stored for up to a maximum of 20 years.

To make sure that we are collecting information accurately, certain regulatory authorities may require access to your medical records. These medical records include electronic records such as the Hampshire Health Records service. Only these regulatory authorities and members of the study team will be able to view your medical records outside of your normal clinical care. Anonymised data may be made available to authorised researchers with a legitimate research interest. Your GP will be informed that you are participating in this study.

What will happen if I decide I do not want to continue in the study?

If you wish to withdraw from the study, all information up to the date of your withdrawal will be used. No further information will be collected about you after withdrawal from the study.

What will happen to the results of the study?

We intend to publish any scientific findings from this study in peer reviewed journals.

Who can I talk to if there is a problem?

If you have any concerns about the study or how it is run, please speak to the researchers who will do their best to answer any questions. If you wish to make a formal complaint, please contact the Patient Support Services Team at Southampton General Hospital, telephone 0238120 6325.

Study contact information

Chief investigator	–	Christopher Kipps, Consultant Neurologist
Study Coordinator	–	Angus Prosser, Research Assistant
Email	–	Angus.prosser@nhs.net
Telephone	–	023 8120 6132

COMPANION INFORMATION SHEET

Brain Imaging in Dementia Study (BRAIID)

Introduction

We would like to invite you to take part in a research study. The aim of this study is to improve our use of brain scans in clinical diagnosis and prognosis (a prediction of the course of a disease) in those with cognitive impairment and dementia.

The study is organised by Dr Christopher Kipps (Consultant Neurologist) and Angus Prosser (PhD student and Research Assistant) at University Hospital Southampton NHS Foundation Trust. The study has been reviewed and approved by the South Central Hampshire A Research Ethics Committee.

Why have I been asked to take part?

You are accompanying / assisting someone who is scheduled to have a scan to look at the activity of their brain (a SPECT or PET scan). Every patient who is booked in to have a SPECT or PET scan will be asked if they would like to take part in the study.

What does the study involve?

- If you are willing to participate, you will be asked to complete a consent form to show that you have agreed to take part.
- We will ask you to help your companion complete a simple 5-minute background questionnaire on the day of consent. Apart from this, we will not collect any data from your companion directly.
- We will access and store data from your companions health care records, including electronic records such as the Hampshire Health Record service and their previous and possible future brain scans. We may contact your companions GP or the physician in charge of their care for some information about them (e.g. relevant medications, past clinical history and clinical tests).
- We would also like to contact you directly throughout the study to find out how your companion is doing throughout the study. Two to four weeks after consent, we will call you to complete a short telephone questionnaire. After this, you will be asked to fill out four questionnaires (Clinical Dementia Rating scale, Cambridge Behavioural Inventory scale, Functional Activities Questionnaire and the Zarit Caregivers Investigation) and provide information on your companion's medications at 6 monthly intervals. You will be able to complete these by post, email, or telephone.

The study will not affect or change any of your companion's normal clinical care. Even if you choose not to participate in the study, your companion's scan will still be completed.

Do I have to take part?

It is up to you whether or not to take part in this study. If you do decide to take part, you can withdraw at any time without giving a reason. If you decide you do not want to take part, it will not affect the standard of care you or your companion receives in any way, and your companion will still receive their scan.

Will my information be kept confidential?

All information that is collected will be kept in the strictest confidence in accordance with the Data Protection Act 1998. All information collected as part of this study will be stored under a code rather than your or your companions name and will have addresses removed. The information will be kept on password protected computers and all information will be kept on secure premises. All data collected will be stored for up to a maximum of 20 years.

To make sure that we are collecting information accurately, certain regulatory authorities may require access to your companion's medical records. These medical records include electronic records such as the Hampshire Health Records service. Only these regulatory authorities and members of the study team will be able to view these medical records outside of normal clinical care. Anonymised data may also be made available to authorised researchers with a legitimate research interest.

What will happen if I decide I do not want to continue in the study?

If you or your companion wishes to withdraw from the study, all information up to the date of withdrawal will be used. No further information will be collected from you post-withdrawal from the study.

What will happen to the results of the study?

We intend to publish any scientific findings from this study in peer reviewed journals.

Who can I talk to if there is a problem?

If you have any concerns about the study or how it is run, please speak to the researchers who will do their best to answer any questions. If you wish to make a formal complaint, please contact the Patient Support Services Team at Southampton General Hospital, telephone 0238120 6325.

Study contact information

Chief investigator	–	Christopher Kipps, Consultant Neurologist
Study Coordinator	–	Angus Prosser, Research Assistant
Email	–	Angus.prosser@nhs.net
Telephone	–	023 8120 6132

CONSULTEE INFORMATION SHEET

Brain Imaging in Dementia Study (BRAIID)

Introduction

We would like to invite you to take part in a research study. The aim of this study is to improve our use of brain scans in clinical diagnosis and prognosis (a prediction of the course of a disease) in those with cognitive impairment and dementia.

The study is organised by Dr Christopher Kipps (Consultant Neurologist) and Angus Prosser (PhD student and Research Assistant) at University Hospital Southampton NHS Foundation Trust. The study has been reviewed and approved by the South Central Hampshire A Research Ethics Committee.

Why have I been asked to take part?

You are accompanying / assisting someone who is scheduled to have a scan to look at the activity of their brain (a SPECT or PET scan). Every patient who is booked in to have a brain SPECT or PET scan will be asked if they would like to take part in the study.

The person you are assisting has been assessed to lack capacity (the ability to make an informed decision) to consent for the study at this time, due to cognitive difficulties related to their illness. Due to this, you have been asked to be a consultee to your companion to assess whether or not you think that they would be willing to participate in the study.

Who can act as a consultee and what is the role of the consultee?

The consultee is someone who knows the person who is lacking capacity in a personal sense. They will usually be a spouse, carer, family member or friend of the individual. The role of the consultee is to inform the study researcher about the participant's wishes and feelings in relation to research, and whether they believe they would be willing to take part in the study.

If you believe your companion would be prepared to take part in the study, you will be asked to complete a consultation form to show that your companion's wishes in regards to research have been discussed.

Do I have to take part?

It is up to you whether or not to act as a consultee. If you decide you do not want to take part, your companion will not be recruited into the study. They will still receive their SPECT scan, and the care they receive will not be affected in any way.

What does the study involve?

- We will contact your companion's GP or the physician in charge of their care for some information about them.
- We will access and store data from your companion's health care records, including electronic records such as the Hampshire Health Record service during the study. This data will include relevant past medical history, medications, cognitive assessments and brain scans.
- We will not contact or collect any data from your companion directly, and the study protocol does not contain any invasive procedures.

Will my companion's information be kept confidential?

All information that is collected will be kept in the strictest confidence in accordance with the Data Protection Act 1998. Information collected as part of this study will be stored under a code rather than your companions name and will have addresses removed. The information will be kept on password protected computers and all information will be kept on secure premises. All data collected will be stored for up to a maximum of 20 years.

To make sure that we are collecting information accurately, certain regulatory authorities may require access to your companion's medical records. These medical records include electronic records such as the Hampshire Health Records service. Only these regulatory authorities and members of the study team will be able to view these medical records outside of normal clinical care. Anonymised data may be made available to authorised researchers with a legitimate research interest. Your companion's GP will be informed that they are participating in this study.

What if believe that my companion no longer wishes to continue in the study?

If you believe your companion wishes to withdraw from the study, please notify the research team. Your companion will then be removed from the study, and no further information about them will be collected. Information up to the date of their withdrawal will be used.

What will happen to the results of the study?

We intend to publish any scientific findings from this study in peer reviewed journals.

Study contact information

Chief investigator	–	Christopher Kipps, Consultant Neurologist
Study Coordinator	–	Angus Prosser, Research Assistant
Email	–	Angus.prosser@nhs.net
Telephone	–	023 8120 6132

Wessex Neurological Centre
Level A, MP 101
Southampton General Hospital
Tremona Road
Southampton
SO166YD

Dr .....
GP address

Date

Dear *Dr* .....

Patient:
Patient Address:
Date of birth:
NHS number:

RE: Brain imaging in dementia study (BRAIID)

Your patient has agreed to participate in the above study. This is an observational multi-centre study of patients with cognitive complaints who have received a single-photon emission computed tomography (SPECT) brain scan. Carers of participants who have had a brain scan are also being asked to take part in the study. A copy of the participant information sheet is enclosed which describes the study in greater detail.

If you would like further information about the study, please do not hesitate to contact me.

Yours sincerely,

Angus Prosser
Research Assistant – Dr Kipps Neurology

Tel: 02381206132
Email: angus.prosser@nhs.net

Enc. Participant information sheet

B.3 BraIID participant consent forms

PARTICIPANT CONSENT FORM
Brain Imaging in Dementia Study (BRAIID)

Please initial end box to indicate consent

I have read and understood the BRAIID participant information sheet Version 1.5 dated 01 February 2017. I have had sufficient time to consider the information and decide whether to take part, and I have had the opportunity to ask any questions and had these questions answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, and without my medical care or legal rights being affected and if I withdraw from the study for any reason, data collected up to my withdrawal will be used.	
I understand that relevant sections of my medical notes and data collected about me in the study may be looked at by individuals from the study site team, certain regulatory authorities or the NHS trust, where it is relevant to my taking part in this research. I give my permission for these individuals to have access to my records.	
I give permission for my relevant clinical data to be obtained and used in this study. I also give permission for the study site team to access my electronic health care records, such as the Hampshire Health Record service.	
I give permission for the study site team to contact me to inform me about upcoming studies for which I may be eligible and that I may be interested in participating in.	
I give permission for the study site team to contact my GP and companion (if recruited) for information about me, and to inform them of my participation in this study.	

Participant Name	Date	Signature
Investigator Name	Date	Signature

Original for the site file, one copy for medical notes and one copy to participant.

COMPANION CONSENT FORM
Brain Imaging in Dementia Study (BRAIID)

Please initial end box to indicate consent

I have read and understood the BRAIID participant information sheet Version 1.5 dated 01 February 2017. I have had sufficient time to consider the information and decide whether to take part, and I have had the opportunity to ask any questions and had these questions answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, and without my own or my companions medical care or legal rights being affected and if I withdraw from the study for any reason, data collected up to the date of my withdrawal will be used.	
I understand that data collected about my companion in the study may be looked at by individuals from the study site team, certain regulatory authorities or the NHS trust, where it is relevant to their taking part in this research. I agree that these individuals can have access to my companion's records.	
I am willing to give follow-up information on my companion and I give permission for the study site team to contact me.	

Companion name:	
Relationship to patient:	
Address:	
Telephone number:	
Signature and Date:	

Investigator name:	
Signature and Date:	

Original for the site file, one copy for medical notes and one copy to companion.

Appendix B

PERSONAL CONSULTEE DECLARATION FORM
Brain Imaging in Dementia Study (BRAIID)

Name of patient	

Please initial end box

I have read and understood the BRAIID consultee information sheet Version 1.5 dated 01 February 2017. I have had sufficient time to consider the information and decide whether my companion would like to take part, and I have had the opportunity to ask any questions and had these questions answered satisfactorily.	
In my opinion I believe the above named person would have no objections to taking part in this research.	
I understand that my companion's participation is voluntary and that I am free to withdraw them at any time, without giving reason, and without my companions medical care or legal rights being affected and if I withdraw them from the study for any reason, data collected up to withdrawal will be used.	
I understand that relevant sections of their medical notes and data collected about them in the study may be looked at by individuals from the study site team, certain regulatory authorities or the NHS trust, where it is relevant to their taking part in this research. I understand their brain imaging data and pathology results will be obtained and used in this study.	
I understand the study site team will access their electronic health care records, such as the Hampshire Health Record service.	
I understand the study site team will contact my companions GP for information about them, and to inform them of their participation in this study.	

Consultee name	
Relationship to patient	
Next of kin?	**YES** **NO**
Signature and Date	

Investigator name	
Signature and Date	

Original for the site file, one copy for medical notes and one copy to consultee.

B.4 BraIID demographics questionnaire

DEMOGRAPHICS QUESTIONNAIRE
Brain Imaging in Dementia Study (BRAIID)

To be completed by (or on behalf of) the patient having the SPECT scan. Please try to answer all questions: write in the blank spaces or circle options that apply to you.

Full name						
Date of birth			**Place of birth**			
Sex	Male	Female	**Handedness**		Right handed	Left handed
First language	English		**Other (specify)**			
Companion / carer						
Their relationship to you?						
Your living arrangements?	Live with partner / family member/ carer			Live alone		
Are you currently	Employed full-time			Employed part-time		
	Not working due to illness			Retired		
Your current / former occupation?						
At what age did you leave school?						
The highest year of education you completed (please circle a number)	Primary school	Secondary school	Sixth Form	University undergraduate	University Post-graduate	
	1 2 3 4 5 6	7 8 9 10 11	12 13	14 15 16	17 18 19 20 21 22 23+	
Have you been a smoker?	Yes	No	**If yes but stopped, when did you stop?**			
What is your weekly alcohol intake (units)			One unit = A small glass of wine Half a pint of beer One shot of spirits			
Have you ever drunk more than 8 units a day regularly?	Yes	No				
Have you noticed problems with your memory or thinking?	Yes	No	**If yes, when did your memory or thinking problems first start?**			

What was your first symptom?			
Have you had any of the following medical problems?	Epilepsy or fits	Parkinson's	Head injury
	Stroke or 'mini-stroke'	Heart attack or angina	High blood pressure
	Diabetes	High Cholesterol	Depression or 'nerves'
Any other medical problems?			
Did either of your parents have any of the following?	Dementia Parkinson's disease Stroke Heart disease Depression	**If you had any brothers or sisters, did they have any of the following?**	Dementia Parkinson's disease Stroke Heart disease Depression
If yes, who?		**If yes, who?**	
Any other serious illness?		**Any other serious illness?**	
Do you have any children?			
Do they or have they had any of the following?	Dementia Parkinson's disease Stroke Heart disease Depression		
Any other serious illness?			
If you have any further comments which you feel are relevant please mention them here:			

Thank you

Appendix B

Please list any medications you are currently taking (please ask for more paper if you need it)	Medication	Times a day	Dose	When started

Thank you

Appendix C

C.1 CADI clinician questionnaires

Perfusion SPECT imaging service improvement study

			A1
Patient name		Patient date of birth	
Clinician name			
Please tick your provisional diagnosis (ONE only)	☐ Alzheimer's disease ☐ Frontotemporal dementia ☐ Vascular dementia ☐ Dementia with Lewy bodies If other:	☐ Mild cognitive impairment ☐ Psychiatric ☐ Dementia – other ☐ Neurology – other	
Please mark on the line how confident you are in this diagnosis	Not confident at all ⟝ Extremely confident		
Please rank up to three OTHER potential diagnoses from most likely to least likely (if relevant)	☐ Alzheimer's disease ☐ Frontotemporal dementia ☐ Vascular dementia ☐ Dementia with Lewy bodies If other:	☐ Mild cognitive impairment ☐ Psychiatric ☐ Dementia – other ☐ Neurology – other ☐ None	

Please return in the enclosed envelope

UHS perfusion SPECT service improvement V2 01 September 2016

Appendix C

Perfusion SPECT imaging service improvement study

Please complete **after** reading the HMPAO-SPECT report, and **return in the enclosed envelope**

			A2
Patient name		Patient date of birth	
Clinician name			
Please tick your provisional diagnosis (ONE only)	☐ Alzheimer's disease ☐ Frontotemporal dementia ☐ Vascular dementia ☐ Dementia with Lewy bodies If other:		☐ Mild cognitive impairment ☐ Psychiatric ☐ Dementia – other ☐ Neurology – other
Please mark on the line how confident you are in this diagnosis	Not confident at all ——— Extremely confident		
Please rank up to three OTHER potential diagnoses from most likely to least likely (if relevant)	☐ Alzheimer's disease ☐ Frontotemporal dementia ☐ Vascular dementia ☐ Dementia with Lewy bodies If other:		☐ Mild cognitive impairment ☐ Psychiatric ☐ Dementia – other ☐ Neurology – other ☐ None
Do you feel the perfusion SPECT imaging contributed to the diagnostic process?	☐ Yes		☐ No
Please mark on the line how helpful you feel the perfusion SPECT was in aiding your diagnosis	Not helpful at all ——— Extremely helpful PTO		

UHS perfusion SPECT service improvement V2 01 September 2016

Please tick the statement that is most applicable	☐ The perfusion SPECT imaging confused my understanding of the patients disease ☐ The perfusion SPECT imaging did not contribute to my understanding of the patients disease ☐ The perfusion SPECT imaging improved my understanding of the patients disease

Appendix D

D.1 Statistical parametric mapping settings

D.1.1 Normalisation

- Estimation options:
    - Template image = Template_8mmCS_A1.img (Dr R. Holmes template)
    - All others at default:
        - Template Weighting Image = 0 files
        - Source Image Smoothing = 8
        - Template Image Smoothing = 0 files
        - Affine Regularisation = *ICBM space template
        - Nonlinear Frequency cutoff = 25
        - Nonlinear Iterations = 16
        - Nonlinear Regularisation = 1
- Writing Options:
    - Preserve = Preserve concentrations
    - Bounding box = 2 x 3 double (-90 -126 -72
      91 91 109)
    - Voxel sizes = [2 2 2]
    - Interpolation = Trilinear
    - Wrapping = no wrap

D.1.2 Smoothing options

- FWHM = [16 16 16]
- All others at default
    - Datatype = SAME
    - Implicit Masking = No

D.1.3 Group analysis

- Design:
  - Two-sample t-test
  - Independence = independent
  - Variance = equal
  - Grand mean scaling = No
  - ANCOVA = No
- Masking:
  - Threshold masking = none
  - Implicit mask = Yes
  - Explicit mask = GMplusWM_final.img (Dr R. Holmes mask)
  - Global normalisation = none
- Contrasts:
  - t-contrasts
  - Type= t-contrast
  - Contrast weights vector = -1 1
- Results box:
  - Apply masking = none
  - P value adjustment to control = none / FWE
  - Threshold = High (0.001), Low (0.05)
  - Extent threshold (voxels) = 100

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