Understanding and improving the diagnosis of dementia with Lewy bodies

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Abstract

Background

Accurate diagnosis of dementia with Lewy bodies (DLB) has important implications for treatment and prognosis, but it is not currently clear how frequently DLB is diagnosed in routine clinical practice, nor how frequently they are assigned an alternative dementia diagnosis.

^{123}I-metaiodobenzylguanidine (MIBG) may be capable of improving DLB diagnostic accuracy but it has not been investigated in clinically representative populations that include patients with comorbidities or interfering medications.

Methods

We conducted a cross-sectional survey of 5 569 patients attending three Psychiatry of Old Age services. From this cohort, 51 DLB and 51 matched non-DLB cases consented to extraction of data relating to the diagnostic process from their clinical case notes.

We enrolled a clinically representative cohort of 17 patients with DLB and 16 with Alzheimer's disease (AD) to a MIBG utility study. Each patient underwent detailed clinical examination, cardiac MIBG and FP-CIT SPECT imaging.

Results

DLB represented 5.6% of dementia cases but prevalence varied across services (3.5-5.9%). DLB cases were often given a different dementia subtype diagnosis (39%) and experienced a longer time from referral to diagnosis (265 days) than non-DLB patients (154 days).

MIBG had a sensitivity and specificity of 71% and 75% for differentiating DLB from AD, but a lower HMR threshold enhanced specificity (100%) without compromising sensitivity. No significant relationships between HMR and either myocardial infarction, or medication prescription, were identified.

Conclusions

Variation in DLB prevalence across services may suggest differences in detection rather than in the true prevalence of the disease. The higher frequency of clinical contacts seen in DLB may provide opportunities to improve both diagnostic accuracy and time to diagnosis.

Our findings support the use of representative cohorts in further MIBG research, particularly in determining appropriate HMR cut-offs. Our finding that three DLB patients had normal MIBG, but abnormal FP-CIT results challenges the Braak hypothesis of DLB pathogenesis.

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Declaration

No portion of the work in this thesis has been submitted in support of an application for another degree or qualification at this or any other university or institute of learning. I confirm this thesis is my own work, and any assistance from others is acknowledged.

Professor John O'Brien (University of Cambridge, Newcastle University (NU)) was the principal investigator of our study of DLB frequency and was responsible for the grant application and ethical approval (including approval from the Confidentiality Advisory Group). The author was responsible for the management of the project under Professor O'Brien's supervision.

The author, together with Sally Barker (Research Assistant, NU) and Neil Fullerton (Clinical Trial Officer, DeNDRoN) screened the clinical notes during Phase 1 of the study. Sally Barker and the author were responsible for recruitment, note screening and data entry, and were assisted by Alison Killen (Research Assistant, NU). Clinical diagnoses were made by the author, Professor O'Brien, Professor Alan Thomas (NU), Professor Ian McKeith (NU), and Dr John-Paul Taylor (NU). The author conducted data analysis under the supervision of Professor O'Brien.

The author managed our MIBG study under the supervision of Professor Thomas, including the development of the project (also supervised by Professor O'Brien and Dr Jim Lloyd) and ethical approval. Professor Thomas provided funding for the project. The author was responsible for recruitment (assisted by DeNDRoN), clinical assessment, patient and carer questionnaires. Helen Kain (Research Support Secretary, NU) provided administrative, participant liaison and data input support. Clinical diagnoses were made by the author, Professor Thomas and Professor O'Brien.

Ms Gemma Roberts (Senior Medical Physicist, Newcastle upon Tyne Foundation Hospitals Trust (NuTH)) calibrated cameras for MIBG use, and with the author performed semiquantitative analysis of MIBG data. Visual analysis of FP-CIT scans was conducted by

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The author is responsible for the writing of this thesis.

Publications

The following publication contains data from Phase 1 of our study of the frequency of DLB cases in NHS clinical services.

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Abbreviations

5-HT_{2A}	5-hydroxytryptamine receptor 2A
A&E	Accident and emergency department
Aβ	Amyloid-β
ACE-R	Addenbrooke's Cognitive Examination (Revised)
ADAS-Cog	Alzheimer's disease assessment scale (cognitive subscale)
ADLs	Activities of daily living
AD	Alzheimer's disease
ARSAC	Administration of Radioactive Substances Advisory Committee
BADLS	Bristol Activities of Daily Living Scale
CAG	Confidentiality Advisory Group
CDR	Clinical Dementia Rating
CFAS	Cognitive Functioning and Ageing Study
CI	Confidence intervals
CNS	Central nervous system
CRF	Clinical Record Form
CSDD	Cornell Scale for Depression in Dementia
CSF	Cerebrospinal fluid
CT	Computerised tomography
CMHT	Community Mental Health Team
DCSF-R	Dementia Cognitive Fluctuations Scale (Revised)
DLB	Dementia with Lewy bodies
EEG	Electroencephalography
EMG	Electromyography
FDG	^{18}F-fluorodeoxyglucose
FP-CIT	*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane single photon emission computer tomography
FTD	Frontotemporal dementia
GP	General practitioner
HMPAO	^{99m}Tc-hexamethyl propyleneamine oxime

HMR	Heart: mediastinum ratio
keV	Kiloelectron volt
IADLS	Instrumental Activities of Daily Living Scale
LBs	Lewy bodies
LEHR	Low energy high resolution collimator
LNs	Lewy neurites
LOR	Lines of response
MBq	Megabecquerels
mCi	Millicuries
MCI	Mild cognitive impairment
MEGP	Medium energy general purpose collimator
mg	Milligrams
MI	Myocardial infarction
MIBG	^{123}I-metaiodobenzylguanidine
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
MSA	Multisystem atrophy
mSv	Millisievert
MTL	Medial temporal lobe
NET	Norepinephrine transporter
NFTs	Neurofibrillary tangles
NIA-AA	National Institute on Aging and Alzheimer's Association (USA)
NICE	National Institute for Health and Clinical Excellence
NIHR	National Institute for Health Research
NHS	National Health Service (United Kingdom)
NMDA	N-methyl-D-aspartate
NPI	Neuropsychiatric Inventory
P-tau	Phosphorylated tau
PET	Positron emission tomography
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Positron emission tomography

POA	Psychiatry of old age
PPMI	Parkinson Progression Marker Initiative
PSP	Progressive supranuclear palsy
RBD	Rapid eye movement sleep behaviour disorder
REC	Research Ethics Committee
REM	Rapid eye movement
ROC	Receiving operating characteristic
ROI	Region of interest
SBR	Specific binding ratio
SD	Standard deviation
SPECT	Single photon emission computerised tomography
SPSS	Statistical Package for Social Sciences
T-tau	Total tau
UK	United Kingdom
UPDRS	Unified Parkinson's Disease Rating Scale (motor subscale)
USA	United States of America
VaD	Vascular dementia
VOI	Volume of interest

Chapter 1

Introduction

Dementia is the leading cause of disability and dependence in older people (Sousa *et al.*, 2009, 2010), and it represents arguably the most significant challenge facing the global health and social care community. An estimated 47 million people worldwide are living with dementia, and this is expected to increase to 75 million by 2030 and 132 million by 2050 (Prince *et al.*, 2015). In the United Kingdom (UK) alone, 6.5% of the population over 65 have dementia (Matthews *et al.*, 2013), and 183 000 new cases develop annually; this is projected to increase as the population ages (Matthews *et al.*, 2016).

Dementia is composed of several subtypes. Alzheimer's disease (AD), vascular dementia (VaD), frontotemporal dementia (FTD), and Lewy body dementia (comprising both dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD)) make up the majority of cases (Stevens *et al.*, 2002).

An important role of clinicians is to determine which dementia subtype applies to the patient presenting to their services. Each subtype has a characteristic combination of signs and symptoms, but these can overlap with each other, and can be complicated both by mixed pathologies and comorbid conditions (Schneider *et al.*, 2009). Even when biomarkers are integrated into the assessment process, every subtype diagnosis is made with at least a degree of uncertainty, as only *post-mortem* examination can definitively confirm the pathology present. In spite of this uncertainty, good clinical practice recommends that a subtype diagnosis is made wherever possible (NICE, 2018), and accuracy of this diagnosis is of great importance as a patient's dementia subtype has implications for management and prognosis.

DLB is one such common dementia subtype with characteristic clinical features (McKeith *et al.*, 2017) and validated biomarkers (McKeith *et al.*, 2007) but with difficulties in detection in routine secondary care (Nelson *et al.*, 2012; Huang and Halliday, 2013) as many cases are missed, often mistaken for AD (Galvin, 2015). Accurate diagnosis of DLB is crucial, however, as DLB is associated with higher rates of neuropsychiatric symptoms, hospitalisation (Mueller *et al.*, 2018), and shorter survival (Price *et al.*, 2017). Accurate recognition reduces likelihood of prescription of

antipsychotic medications, to which patients with DLB can experience severe sensitivity (Aarsland *et al.*, 2005).

This thesis will therefore examine DLB diagnostic practice in routine clinical services and the factors behind DLB detection. It will describe a large epidemiological study investigating DLB diagnostic rates in the UK National Health Service (NHS) secondary care services and how patients' contact with these services differs from that of patients with other dementia subtypes. The thesis will then explore the role of several biomarkers in optimising the clinical detection in DLB, before investigating the utility of one such biomarker, ^{123}I-metaiodobenzylguanidine (MIBG) cardiac scintigraphy, in differentiating DLB from AD. This first chapter will provide an overview of both DLB and AD, the subtype for which DLB is likely most commonly mistaken.

1.1 Dementia with Lewy bodies

1.1.1 Clinical features

Dementia is defined as decline in cognitive function that interferes with a person's ability to undertake their usual activities, which is not explained by delirium or another psychiatric disorder (McKhann *et al.*, 2011). DLB diagnosis is made on the basis of recognition of characteristic signs and symptoms of disease in someone with dementia.

The neuropsychological profile of this cognitive decline differs in DLB from that seen in AD. Patients in the early stages of DLB experience greater impairment in visuospatial, attentional and frontal-executive function, with greater preservation of memory than those with early AD (Metzler-Baddeley, 2007).

Pronounced fluctuations in cognition, arousal and functioning, superimposed upon this progressive cognitive decline, are often seen in DLB and represent a characteristic diagnostic feature of the disease (McKeith *et al.*, 2005, 2017). They are thought to arise as a consequence not of specific structural changes, but rather disruptions in functional networks (Peraza *et al.*, 2014). The presence of these fluctuations can have a significant impact upon patients and their carers (Lee *et al.*, 2013). Although variations in alertness and concentration in DLB have been demonstrated in computerised tests of reaction times (Ballard, *et al.*, 2001), accurate detection of fluctuations in the clinic have proved challenging; clinicians assessing the symptom show low inter-rater reliability (Lee *et al.*, 2012).

Another important symptom of DLB is the presence of recurrent complex visual hallucinations. The aetiology of this symptom is poorly understood (Boeve *et al.*, 2003) but affect up to 80% of patients with DLB (Aarsland *et al.*, 2001) and are associated with significant impairment in quality of life (Boström and Jönsson, 2007). The hallucinations themselves are often recurrent, detailed representations of small children or animals and although the reaction of the patient can vary from mirth and indifference to considerable anguish (McKeith *et al.*, 2005), caregivers can experience significant distress as a consequence of this symptom (Ricci *et al.*, 2009). Patients with DLB can also experience other neuropsychiatric features, such as depression, anxiety, apathy, delusions and hallucinations in other modalities, but none of these carry the same diagnostic specificity as visual hallucinations (McKeith *et al.*, 2017).

Spontaneous motor features of parkinsonism (bradykinesia, rigidity, rest tremor and gait and postural reflex abnormalities) are observed in up to 85% of patients with DLB (Ferman *et al.*, 2006). The phenotype of postural instability and gait disturbance is more commonly seen in DLB than tremor-dominant disease when compared with PD patients (Burn *et al.*, 2006), but rest tremor, action tremor, bradykinesia, decreased facial expression and rigidity were observed as the features capable of best discriminating DLB from AD in a pathologically confirmed sample (Ballard *et al.*, 1997).

In recent years rapid eye movement (REM) sleep behaviour disorder (RBD) has emerged as a strong predictor of DLB at *post-mortem* (Ferman *et al.*, 2011), preceding dementia or parkinsonism by up to 30 years (Boeve *et al.*, 2003). It is also closely associated with other synucleinopathies, such as Parkinson's disease (PD), PD dementia (PDD) and multisystem atrophy (MSA) (Iranzo *et al.*, 2013). RBD is characterised by a loss of physiological muscle atonia during REM sleep, manifesting as enactment of the patient's dreams; punching, kicking or vocalising. This may result in injury to the patient or bed partner (Boeve, 2010). RBD is ideally confirmed using polysomnography (PSG) (McKeith *et al.*, 2017) but a validated questionnaire for use in the clinical setting is associated with a high diagnostic sensitivity and acceptable specify for RBD (Boeve *et al.*, 2011).

Approximately half of DLB patients administered antipsychotic agents suffer from sensitivity reactions (Aarsland *et al.*, 2005), which can range in severity from sedation, increased confusion, rigidity and immobility to markedly reduced survival (McKeith, Ballard and Harrison, 1995). The last decade has seen the antipsychotic prescription in patients with dementia half, but 11% still

receive these agents (Donegan *et al.*, 2017) and as a pronounced and iatrogenic clinical feature, the risk of neuroleptic sensitivity remains an important rationale for the detection of DLB in the clinical setting (McKeith *et al.*, 2017).

Several clinical signs and symptoms, other than those already discussed, are associated with DLB, but their high prevalence in dementia populations mean that these features lack the specificity to carry significant weight towards DLB diagnosis (McKeith *et al.*, 2017). These include frequent falls, transient loss of consciousness, delusions, depression, anxiety and autonomic dysfunction, which can manifest as constipation, urinary incontinence or orthostatic hypotension (Horimoto *et al.*, 2003). These symptoms direct clinicians towards useful management strategies and therefore require vigilance.

Patients with DLB can therefore exhibit a range of symptoms in addition to the cognitive and functional decline associated with dementia itself. Several of these, notably cognitive fluctuations, visual hallucinations, spontaneous features of parkinsonism and RBD, are of particular use in the clinical setting by virtue of their prevalence and specificity in differentiating DLB from other dementia subtypes.

1.1.2 Diagnostic criteria

Diagnostic criteria for DLB have provided a framework for detection of the disease in both research and clinical settings by operationalising these characteristic clinical features and integrating validated biomarkers, an approach consistent with that employed in other dementia subtype criteria (McKhann *et al.*, 2011). Successive revisions of criteria have aimed to enhance sensitivity and reflect important developments in the evidence base surrounding DLB.

Criteria published as part the third report of the International DLB Consortium ("*the third consensus criteria"*) were observed to demonstrate superior sensitivity to their preceding criteria (McKeith *et al.*, 1996) in a *post-mortem* validation study (McKeith *et al.*, 2000b); however, meta-analysis has since shown that this improvement in sensitivity has been at the expense of specificity (Rizzo *et al*., 2017). The third consensus criteria, displayed in *Figure 1.1*, allow diagnosis of "*probable*" or "*possible*" DLB based on different combinations of "*core*" and "*suggestive*" clinical symptoms.

Although since revised as part of the fourth report of the International DLB Consortium ("*the fourth consensus criteria")* (McKeith *et al.*, 2017)*,* the third consensus criteria were the prevailing criteria at the time of the development and analysis of the studies comprising this thesis, and therefore incorporated in their design.

Figure 1.1 Revised ("third consensus") criteria for the clinical diagnosis of DLB (McKeith et al., 2005)

Central feature (essential for diagnosis of probable or possible DLB)
Dementia
Core features*
Fluctuating cognition with pronounced variations in attention and alertness
Recurrent visual hallucinations that are typically well formed and detailed
Spontaneous features of parkinsonism
Suggestive features*
REM sleep behaviour disorder
Severe neuroleptic sensitivity
Low dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET imaging
Supportive features (commonly present but not proven to have diagnostic specificity**)**
Repeated falls and syncope
Transient, unexplained loss of consciousness
Severe autonomic dysfunction e.g., orthostatic hypotension, urinary incontinence Hallucinations in other modalities
Systematized delusions
Depression
Relative preservation of medial temporal lobe structures on CT/MRI scan
Generalized low uptake on SPECT/PET perfusion scan with reduced occipital activity
Abnormal (low uptake) MIBG myocardial scintigraphy
Prominent slow wave activity on EEG with temporal lobe transient sharp waves
*A diagnosis of *probable* DLB can be made in the presence of at least two core features, or one core feature and one suggestive feature. A diagnosis of *possible* DLB can be made in the presence of one core feature, or in the presence of one or more suggestive features.

Figure 1.2 Revised ("fourth consensus") criteria for the clinical diagnosis of probable and possible DLB (McKeith et al., 2017)

Essential
Dementia
Core clinical features *
Fluctuating cognition with pronounced variations in attention and alertness
Recurrent visual hallucinations that are typically well formed and detailed
REM sleep behaviour disorder, which may precede cognitive decline
One or more spontaneous cardinal features of parkinsonism
Supportive clinical features
Severe sensitivity to antipsychotic agents; postural instability; repeated falls; syncope or other transient episodes of unresponsiveness; severe autonomic dysfunction, e.g., constipation, orthostatic hypotension, urinary incontinence; hypersomnia; hyposmia; hallucinations in other modalities; systematized delusions; apathy, anxiety, and depression
Indicative biomarkers*
Reduced dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET
Abnormal (low uptake) ^{123} iodine-MIBG myocardial scintigraphy.
Polysomnographic confirmation of REM sleep without atonia
Supportive biomarkers
Relative preservation of medial temporal lobe structures on CT/MRI scan
Generalized low uptake on SPECT/PET perfusion/metabolism scan with reduced occipital activity ± the cingulate island sign on FDG-PET imaging
Prominent posterior slow-wave activity on EEG with periodic fluctuations in the pre-alpha/ theta range.
*A diagnosis of *probable* DLB can be diagnosed on the basis of two or more core clinical features, or one core clinical feature and one indicative biomarker. *Possible* DLB can be diagnosed on the basis of one core clinical feature, or if one or more indicative biomarkers are present in the absence of any core clinical features.

The fourth consensus criteria *(Figure 1.2)* have maintained the fundamental structure of the third consensus criteria. They aimed to enhance sensitivity of diagnosis but have not yet been compared with neuropathological data. These criteria increased the diagnostic weighting of RBD, including it as a core clinical feature, and replaced the suggestive symptom category of the third

criteria with a group of "*indicative"* biomarkers; reduced dopamine transporter uptake in basal ganglia demonstrated by single photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging, reduced uptake on MIBG myocardial scintigraphy, and PSG confirmation of REM sleep without atonia.

Both criteria detail the distinction between DLB and PDD, based on the temporality of dementia relative to parkinsonian symptoms. Where dementia occurs concurrently, or shortly before the onset of parkinsonism, a DLB diagnosis is appropriate, whereas, PDD refers to the onset of dementia in the context of established PD. This distinction is operationalised for research by the "*one year rule*", in which DLB, rather than PDD, is diagnosed when cognitive symptoms precede, or occur within a year of the emergence of parkinsonian symptoms (McKeith *et al*., 2017).

1.1.3 Biomarkers

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pharmacologic responses to a therapeutic intervention, or, indicators of pathogenic processes (Biomarkers Definitions Working Group, 2001). Biomarkers are of increasing importance as research frameworks begin to move away from a syndromic definition of dementia subtypes (that is, on the basis of signs and symptoms), to a more biological construct that allows more precise characterisation of disease processes. In of DLB, biomarkers also play an important clinical role in case detection (Jack *et al.,* 2018).

Both the third and fourth consensus criteria incorporate several biomarkers, of which three may be combined with clinical symptoms to arrive at a possible or probable DLB diagnosis. Of these, two are nuclear medicine techniques; low dopamine transporter uptake in the basal ganglia, typically determined by ^{123}I-2β- carbometoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl) nortropane SPECT (FP-CIT), and reduced myocardial denervation measured by MIBG uptake scintigraphy. Both are discussed in detail in Chapter 3. The third technique is PSG, used to confirm the presence of RBD. PSG incorporates data from several physiological parameters, most notably electroencephalography (EEG) used to determine the high frequency, low amplitude activity suggestive of REM sleep, and electromyography (EMG), used to measure skeletal muscle activity. REM sleep without the presence of atonia is diagnostic of RBD, and even in the absence of other

core features is highly predictive of a synucleinopathy in a patient with dementia (Boeve *et al.*, 2013).

1.1.4 Neuropathology

Although diagnostic criteria maintain a focus on clinical symptoms, a definitive diagnosis of DLB can only be made upon *post-mortem* examination, when the pathological hallmarks of the disease, Lewy bodies (LBs) and Lewy neurites (LNs), can be observed. Both LBs and LNs contain the aggregates of α-synuclein, a synaptic protein, and can therefore be detected using techniques such as staining with anti-α-synuclein antibodies (Gómez-Tortosa *et al.*, 2000). In addition to LB and LN pathology, the characteristic substrates of AD, neurofibrillary tangles (NFTs) and amyloid-beta (Aβ), are seen in a high proportion of DLB cases (McKeith *et al.*, 2005). The extent of this pathology can greatly influence the clinical phenotype (Thomas *et al.*, 2018b).

LBs and LNs are distributed throughout the central and peripheral nervous system in patients with DLB (Beach *et al.*, 2010), as well as in other dementia subtypes such as AD and in a proportion of healthy individuals (Zaccai *et al.*, 2015). Clinicopathological diagnosis therefore depends on the distribution of AD pathology (Montine *et al.*, 2012) as well as that of both LBs and LNs.

Figure 1.3 Assessment of the likelihood that the pathologic findings are associated with a typical, dementia with Lewy bodies, clinical syndrome (McKeith et al., 2017)

	Alzheimer disease neuropathological change (NIA-AA)		
Lewy related pathology	Low (Braak 0-II)	Intermediate (Braak III – IV)	High (Braak IV -VI)
Diffuse (neocortical)	High	High	Intermediate
Limbic (transitional)	High	Intermediate	Low
Brainstem-predominant	Low	Low	Low
Amygdala-predominant	Low	Low	Low
Olfactory bulb only	Low	Low	Low

Five main patterns of distribution of LBs and LNs are recognised, each carrying varying likelihoods that pathological findings correspond to clinically apparent DLB (McKeith *et al.*, 2017) (*Figure 1.3*). Of these five patterns, diffuse distribution of LBs and LN throughout the neocortex is the most common in DLB (Zaccai *et al.*, 2015); where observed in this manner there is a high likelihood of

expression of clinical DLB symptoms in all but cases with advanced AD disease. The probability of a DLB phenotype in a patient with limbic LB/LN pathology is associated with lower burdens of AD pathology.

Our understanding of the pathogenesis of DLB is largely derived from neuropathological PD research. Braak *et al.* (2003) observed the distribution of α-synuclein in PD patients with varying disease severity, as well as in asymptomatic subjects in whom LB/LN pathology had been identified *post-mortem*. A uniform, predictable spread of pathology conforming to six stages was devised, hypothesised to start in vulnerable "*sentinel*" structures (such as the olfactory bulb and the dorsal motor nucleus of the vagal nerve) before ascending through the pons, midbrain, subcortical structures and neocortex. These stages were sequential; neocortical disease, representing the final symptomatic stages (stages 5 and 6) was only observed in patients in whom there was also evidence of pathology in lower structures, representing stages 1 to 4 and consistent with asymptomatic or mild disease. However, patterns of α-synuclein spread consistent with this "*Braak hypothesis*" have been observed in around only half of patients with clinical LB pathology in a community-based sample (Zaccai *et al.*, 2015). This is further supported by the proportion of DLB patients with neocortical LB pathology that fail to demonstrate findings associated with striatal pathology, as would be expected from the Braak hypothesis; 15-20% never develop parkinsonian symptoms (Ferman *et al.*, 2006; Walker *et al.*, 2016) and 10% have negative FP-CIT scans *antemortem* (Thomas *et al.*, 2017a). This has led to suggestions that the mechanisms underpinning pathogenesis are more complex than previously suggested by the Braak hypothesis, and that both α-synuclein spread and neuronal death may be subject to cell-mediated factors capable of varying between different anatomical regions (Surmeier, Obeso and Halliday, 2017).

1.1.5 Disease progression

There is increasing evidence that the progression of DLB is both heterogeneous and distinct from that seen in other dementia subtypes (Cercy and Bylsma, 1997; Mueller *et al.*, 2017). One possible reason for this heterogeneity may be the effect of varying burdens of pathology, as mixed DLB/AD cases appear to deteriorate more rapidly than those with pure LB pathology (Olichney *et al.*, 1998; Kraybill *et al.*, 2005; Nedelska *et al.*, 2015).

A five-year prospective cohort study found that patients with DLB demonstrated a greater annual decline in MMSE (4.4 points) than those with AD (3.2 points) (Rongve *et al.*, 2016). This was supported by a larger retrospective analysis of DLB patients across 18 countries which found than MMSE score declined more rapidly in DLB than both AD and PDD (Kramberger *et al.*, 2017).

This cognitive decline is mirrored by a more rapid deterioration in functional ability; DLB patients experience higher levels of disability (Boström *et al.*, 2007), shorter time from presentation to nursing home admission (Rongve *et al.*, 2014) and lower overall quality of life (Boström and Jönsson, 2007).

DLB also appears to be associated with a more complex, extensive course of contact with healthcare services than other dementia subtypes. A retrospective analysis of healthcare records found that DLB patients were admitted to hospital more frequently, and for longer periods of time, than both AD patients and the general elderly population (Mueller *et al.*, 2018). Higher rates of comorbidities, such as stroke, migraine and depression have also been observed in DLB groups (Fereshtehnejad *et al.*, 2014). These factors may contribute to the higher rates of mortality in DLB patients; one retrospective naturalistic cohort study observed that the time between initial presentation and death was a median 3.72 years in DLB patients, but 6.95 years in AD patients, independent of age, sex and antipsychotic prescription (Price *et al.*, 2017).

1.1.6 Epidemiology

DLB is frequently cited as the second most common neurodegenerative dementia but there is little consensus regarding the prevalence and incidence of the disease. Neuropathological evidence of DLB is observed in 15 – 30% of patients with dementia (Oinas *et al.*, 2009; Jellinger and Attems, 2011) but clinical signs and symptoms are seen in a smaller proportion of patients *antemortem*. A meta-analysis of 31 epidemiological studies observed DLB in 4.2% of dementia cases in the community and 7.5% of those in secondary services and reported an incidence of the disease of 0.87 per 1 000 patient-years (Vann Jones and O'Brien, 2014).

Among the reasons that DLB prevalence in clinical studies may vary from those in neuropathological studies may be difficulty in diagnosing the disorder in routine practice. Delays in diagnosis often occur and patients may be assigned a different dementia subtype before later

revision to DLB (Galvin, 2015). The clinical epidemiology of DLB, and the detection of the disease in routine clinical practice, is discussed in detail in Chapters 2, 4, 5, and 6.

1.1.7 Management

It is not currently possible to stop or slow down the accumulation of DLB pathology, but several pharmacological agents are in routine use for the symptomatic treatment of DLB. Non-pharmacological treatments, such as interventions to improve gait and swallowing in DLB patients have been investigated with encouraging results (Logemann *et al.*, 2008; Rochester *et al.*, 2009), but a recent systematic review failed to identify any studies of sufficient quality to be included in meta-analysis (Connors *et al.*, 2017).

1.1.7.1 Cognitive symptoms

Cholinergic deficits are seen earlier, and at a greater severity, in DLB than AD, and in the former are associated with both cognitive impairment and visual hallucinations (Tiraboschi *et al.*, 2002). The acetylcholinesterase inhibitors donepezil and rivastigmine therefore form the cornerstones of DLB management. Three randomised controlled trials (RCTs) have demonstrated the efficacy of donepezil and rivastigmine in the treatment of DLB (McKeith *et al.*, 2000a; Mori *et al.*, 2012; Ikeda *et al.*, 2015). Meta-analysis of these studies (Stinton *et al.*, 2015) indicated that both donepezil and rivastigmine were associated with improvements in global outcome measures. Those administered donepezil in these RCTs demonstrated a mean increase of 1.26 points on MMSE compared to those receiving placebo, and improvements in attention following rivastigmine prescription have also been observed (McKeith *et al.*, 2000a). Both agents have shown efficacy in treating neuropsychiatric symptoms (Stinton *et al.*, 2015), particularly apathy, anxiety, hallucinations and delusions (McKeith *et al.*, 2000a). Improvements in cognitive fluctuations, sleep disturbances, and psychiatric symptoms have been reported with a third acetylcholinesterase inhibitor, galantamine (Edwards *et al.*, 2007), but the agent has thus far only been investigated using an open-label design.

Side effects to acetylcholinesterase inhibitors occur up to three quarters of patients with DLB (Bhasin *et al.*, 2007) but these are usually of mild severity; trial dropout rates were no greater in patients with DLB treated with donepezil than those with placebo (Stinton *et al.*, 2015).

Two RCTs have investigated the use of the N-methyl-D-aspartate (NMDA) receptor antagonist memantine in mixed DLB and PDD samples (Aarsland *et al.*, 2009; Emre *et al.*, 2010). Meta-analysis suggests that memantine offered no superiority over placebo in cognition, neuropsychiatric symptoms, or functioning, but did demonstrate improvements in continuous measurement of global assessment (Stinton *et al.*, 2015). Secondary analysis of these studies has, however, suggested that memantine is associated with improvements in sleep behaviour (Larsson *et al.*, 2010) and aspects of quality of life (Larsson *et al.*, 2011).

1.1.7.2 Parkinsonism

Much as in PD, the treatment of parkinsonian symptoms in DLB is primarily with levodopa. Improvements in motor function are seen in around a third of DLB patients treated with levodopa (Bonelli *et al.*, 2004; Molloy *et al.*, 2005; Goldman *et al.*, 2008), a response rate lower than that seen in both PD (80-90%) (Hughes *et al.*, 1993; Lucetti *et al.*, 2010) and PDD (65-70%) (Stinton *et al.*, 2015). Although well tolerated by the majority of patients (Molloy *et al.*, 2005), one third experience an escalation in psychotic symptoms (Goldman *et al.*, 2008). Agents such as amantadine, rotigotine and selegiline are routinely prescribed to patients with PD, but there is little evidence to support their use in DLB (Stinton *et al.*, 2015).

1.1.7.3 RBD, psychosis and depression

In both patients with and without cognitive impairment, treatment of RBD focuses on minimising the risk of injury rather that stopping dream enactment itself (Boeve, 2010) and clonazepam and melatonin are the mainstays of management. Neither have been investigated substantially in DLB (Stinton *et al.*, 2015), but the more favourable side effect profile of melatonin favours its use over clonazepam in older patients.

Psychotic symptoms such as hallucinations and delusions are common in DLB, but management is complicated by both the limited efficacy of antipsychotics, and the risk of pronounced adverse reactions to such agents (McKeith, Ballard and Harrison, 1995; Ballard *et al.*, 1997; Aarsland *et al.*, 2005). Cholinesterase inhibitors therefore represent the first-line management of psychosis in DLB. Quetiapine is favoured over neuroleptics with strong dopamine 2 receptor antagonism, such as typical antipsychotics and the atypical agents olanzapine and risperidone (Boot *et al.*, 2013), but was not associated with an improvement in neuropsychiatric, cognitive, functional or motor symptoms, nor clinician's impression of change in a placebo-controlled trial. Clozapine has

demonstrated the ability to diminish psychotic symptoms in PD in two open-label trials (Factor *et al.*, 2001; Morgante *et al.*, 2004) and one RCT (Pollak *et al.*, 2004), but has not been investigated in DLB populations. Although pimavanserin, a selective serotonin 5-hydroxytryptamine receptor 2A ($5\text{-}HT_{2A}$) inverse agonist, has demonstrated efficacy in reducing neuropsychiatric symptoms at six weeks in both PD psychosis (Cummings *et al.* 2014) and AD psychosis (Ballard *et al.* 2018), there have been no published studies investigating its use in DLB patients, and concerns regarding the safety of pimavanserin have recently arisen (Webster, 2018).

DLB diagnosis therefore guides clinicians towards suitable management strategies and necessitates consideration of important adverse effects. A paucity of robust evidence surrounds the treatment of symptoms such as depression, and the role of non-pharmacological treatments. Systematic reviews have noted the importance in optimising DLB diagnosis in recruiting suitable cohorts for further research into DLB therapies (Stinton *et al.*, 2015; Connors *et al.*, 2017).

1.1.8 Summary

DLB is a common dementia subtype characterised by a tetrad of symptoms that include cognitive fluctuations, visual hallucinations, spontaneous parkinsonism and RBD. The presence of combinations of these symptoms, together with biomarkers, guide clinicians towards a probable or possible diagnosis of DLB. Definitive diagnosis, however, can only occur *post-mortem*, when the LNs and LBs characteristic of the disease can be identified. These findings are often seen in combination with AD pathology, which may make detection of some symptoms more difficult and contribute towards the lack of consensus between pathological and clinical epidemiological studies. Accurate *antemortem* diagnosis is nonetheless crucial, both in guiding clinicians to appropriate management strategies, and in providing disease-specific information to patient and carer; this may include the more rapid rates of cognitive and functional decline and higher mortality rates seen in DLB than in other subtypes.

1.2 Alzheimer's disease

AD is the most common dementia subtype, comprising 50-70% of patients with dementia (Lobo *et al.*, 2000; Reitz, Brayne and Mayeux, 2011). As already discussed, DLB cases often exhibit AD neuropathology *(Chapter 1.1.4),* potentially influencing the clinical presentation (Tiraboschi *et al.*, 2006), and patients with DLB are often diagnosed with AD prior to subsequent revision (Galvin *et*

al., 2010). This section therefore aims to provide a brief overview of AD to further contextualise discussion surrounding the clinical detection of DLB.

1.2.1 Clinical features

AD is characterised by an insidious onset of progressive cognitive impairment, with initial and most prominent deficits seen most commonly in episodic memory (McKhann *et al.*, 2011). Non-amnestic presentations occur less frequently, with disturbances in language, visuospatial ability or executive function. Neuropsychiatric symptoms occur less frequently than in DLB (Hanyu *et al.*, 2009), but depression and apathy are common in the early stages of AD, and other symptoms such as agitation, delusions and hallucinations may occur in the later stages of the disease (Lyketsos *et al.*, 2011).

1.2.2 Diagnostic criteria

Like DLB, a definite diagnosis of AD can only be made *post-mortem.* Diagnostic criteria for AD (McKhann *et al.*, 2011) were developed by the United States of America (USA) National Institute on Aging and Alzheimer's Association (NIA-AA). The criteria share many characteristics of those demonstrated by the third and fourth consensus criteria for DLB (McKeith *et al.*, 2005, 2017), classifying combinations of clinical observations to arrive at a probable or possible diagnosis, reflecting the likelihood of the presence of AD neuropathology. Probable criteria for AD are shown in *Figure 1.4.* A possible diagnosis is made on the basis of either an atypical, rather than insidious, clinical course, or the presence of signs or symptoms associated with other dementia subtypes; this includes the core symptoms characteristic of DLB (McKhann *et al.*, 2011).

1.2.3 Biomarkers

NIA-AA criteria included a separate diagnostic category of *probable AD with evidence of the AD pathophysiological process* in which biomarkers are combined with the criteria for probable AD to reflect a higher certainty that the dementia syndrome is related to neuropathological AD (McKhann *et al.*, 2011). These include cerebrospinal fluid (CSF) biomarkers and imaging biomarkers.

1.2.3.1 CSF biomarkers

Three main CSF biomarkers have been identified and tested; the 42-aminoacid form of Aβ ($A\beta_{42}$), total tau (T-tau) and phosphorylated tau (P-tau). $A\beta_{42}$ reflects the cortical deposition of amyloid plaques, while T-tau and P-tau are measures of neuronal and axonal damage and degeneration. T-tau is recognised as a non-specific marker of neuronal injury, when compared with P-tau, which is more specific to AD (Jack *et al.,* 2018). All three markers individually demonstrate the ability to distinguish AD from healthy controls (Olsson *et al.*, 2016), but achieve higher levels of sensitivity (87%) and specificity (84%) when used in combination (Ferreira *et al.*, 2014). Their utility is lower in distinguishing AD from other dementia subtypes (Olsson *et al.*, 2016), and $A\beta_{42}$, T-tau and P-tau demonstrate value in early disease, discriminating between mild cognitive impairment (MCI) due to AD and stable MCI (Olsson *et al.*, 2016).

Figure 1.4 NIA-AA diagnostic guidelines for probable Alzheimer's disease (McKhann et al., 2011)

Meets criteria for dementia
Evidence of cognitive decline in two or more cognitive domains
Significant interference in ability to function at work or in usual daily activities
AND demonstrates all of the following characteristics;
Insidious onset
Clear-cut history of worsening of cognition by report or observation
AND demonstrates initial and most prominent cognitive deficits in;
Episodic memory (*amnestic* presentation)
Language, visuospatial ability or executive function (*non-amnestic* presentation)
AND the absence of evidence for;
Substantial concomitant cerebrovascular disease
Core features of Dementia with Lewy bodies
Prominent features of behavioural variant frontotemporal dementia
Prominent features of semantic variant primary progressive aphasia or non- fluent/agrammatic variant primary progressive aphasia;
Evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.

Routine use of CSF biomarkers is, however, complicated by variation in how individual studies establish cut-offs between AD patients and controls, partly due to variation in collection, processing and analysis of samples (Mattsson *et al.*, 2013). This has led to the creation of a global quality control programme to standardise practice across sites (Blennow, Zetterberg and Fagan, 2015).

1.2.3.2 Imaging biomarkers

Like CSF biomarkers, imaging biomarkers for AD reflect both the amyloid deposition (detected with PET amyloid imaging) and downstream degeneration (through atrophy on structural imaging and reduced regional metabolism with ^{18}fluorodeoxyglucose (FDG) uptake on PET) characteristic of AD pathology.

The development of the ^{11}C-labelled PET ligand Pittsburgh Compound-B (PiB), and subsequently ^{18}F-labelled ligands such as florbetapir, flutametamol and florbetaben, have allowed detection of amyloid pathology *in vivo* (Klunk *et al.*, 2004). The diffuse, asymmetric pattern of high tracer uptake seen in the prefrontal cortex, precuneus and posterior cingulate, followed by the lateral parietal, lateral temporal cortex, and striatum reflects the distribution of amyloid plaques observed *post-mortem* (Braak *et al.*, 1999). Several studies have observed an excellent sensitivity (>90%) and good specificity (>80%) for distinguishing patients with AD from both healthy controls and patients with other dementia subtypes (Rowe *et al.*, 2010; Rabinovici *et al.*, 2011; Morris *et al.*, 2016). However, the relationship between amyloid deposition and dementia weakens with age, and around 23% of cognitively healthy individuals have amyloid pathology *post-mortem* (Savva *et al.*, 2009). The lower positive predictive value of an amyloid scan in older individuals may mean that such scans are more helpful in ruling out AD (given its excellent sensitivity) rather than in confirming the presence of an AD pathophysiological process.

The structural imaging methods, computed tomography (CT) and magnetic resonance imaging (MRI) can detect the early degenerative changes of AD seen in medial temporal lobe (MTL) structures. MTL atrophy distinguishes AD from healthy controls in with greater than 85% accuracy (Scheltens *et al.*, 2002), and is correlates with both the severity cognitive decline and of AD neuropathology.

FDG PET is used to detect areas of hypometabolism consistent with dysfunction and neurodegeneration. In AD, this is observed in temporo-parietal areas, particularly the angular

gyrus, the cuneus and the posterior cingulate cortex with relative preservation of metabolism in the basal ganglia, motor cortex and visual cortex (Herholz, 2011). This method has demonstrated good sensitivity (85%) and excellent specificity (90%) in distinguishing AD from healthy controls (O'Brien *et al.*, 2014a). ^{99m}Tc-hexamethyl propyleneamine oxime (HMPAO) SPECT, which detects areas of regional neurodegeneration by measurement of cerebral blood flow, is listed in best practice guidelines as a second line investigation in dementia subtype diagnosis (NICE, 2018), but is not specified as an AD biomarker in NIA-AA criteria (McKhann *et al.*, 2011). Both FDG PET and HMPAO are discussed further in Chapter 2.

1.2.4 Neuropathology

The main pathological substrates in AD are neurofibrillary tangles (NFTs) and amyloid-beta (Aβ) plaques, which are seen in association with neuronal degeneration and death.

NFTs are intracellular aggregations of the protein tau, which in its physiological function has a role in maintaining structural cellular integrity through stabilisation of microtubules. Hyperphosphorylation of tau prevents its binding to microtubules and leads to aggregation into insoluble NFTs, which impair neuronal and synaptic function. Beginning in the transentorhinal cortex, NFT pathology appears to develop in a largely uniform manner, spreading to the hippocampus, limbic system and cortex (Braak *et al.*, 2006). The degree of tau pathology burden correlates closely with AD disease severity (Nelson *et al.*, 2012; Ossenkoppele *et al.*, 2016).

Aβ plaques are formed when the physiological processing of amyloid precursor protein (APP) is disrupted by the pathological cleavage catalysed by one or more enzymes, in what has become known as the "*amyloid cascade*" hypothesis; accumulation of insoluble Aβ then triggers plaque and NFT formation, leading to cell dysfunction and death (Reitz, 2012). In contrast to the pattern of NFT development, plaques are first observed in the neocortex before proceeding in a caudal direction to allocortical and brainstem structures (Brettschneider *et al.*, 2015).

1.2.5 Management

No disease-modifying therapies for AD are currently available, and recommended treatment is primarily with the acetylcholinesterase inhibitors, donepezil, rivastigmine and galantamine, and the NMDA receptor antagonist memantine (O'Brien *et al*., 2017; NICE, 2018). Meta-analysis of ten randomised, double blind placebo-controlled trials found that prescription of one of these

medications in patients with mild and moderate AD was associated with improvements, albeit with small effect sizes, in global clinical state, activities of daily living (ADLs) and behaviour (Birks, 2006). Symptomatic benefits in cognition were also seen, equivalent to 1.4 points on MMSE and 2.7 points on the Alzheimer's disease assessment scale cognitive subscale (ADAS-Cog).

1.2.6 Summary

AD is the most common dementia subtype, characterised by an insidious onset of cognitive impairment. This is typically most pronounced in the domain of episodic memory, but a smaller proportion of patients my present with deficits in language, visuospatial ability, or executive function. Like in DLB, diagnostic criteria for AD attempt to combine clinical observations with the neuropathological changes seen in AD, but definitive diagnosis cannot be made *antemortem*, even in the presence of imaging and CSF biomarkers closely associated with amyloid deposition and neuronal degeneration.

Chapter 2

Understanding DLB detection in clinical services

2.1 Introduction

Understanding and optimising the clinical diagnosis of DLB is important. Identification of DLB directs clinicians' attention towards the detection and treatment of symptoms associated with the disease; for example, dopamine replacement therapy for parkinsonian symptoms (Bonelli *et al.*, 2004; Onofrj *et al.*, 2013), or clonazepam for RBD (Massironi, Galluzzi and Frisoni, 2003). Similarly, a DLB diagnosis should direct clinicians away from harmful management strategies like antipsychotic prescription, capable of producing fatal reactions to in patients with DLB (McKeith, Ballard and Harrison, 1995). Detection is also crucial to providing the patient and carer with accurate and evidence-based information on the disease itself, its symptoms, treatments and prognosis. Outcomes associated with DLB, such as increased rates of hospitalisation (Hanyu *et al.*, 2009), nursing home admission (Rongve *et al.*, 2014) and carer stress (Leggett *et al.*, 2011; Lee *et al.*, 2013) are important points of discussion between clinicians, patients and carers. Despite this importance of recognition, and the existence of validated diagnostic criteria (McKeith *et al.*, 2005, 2017), initial misdiagnosis of DLB is a common experience (Galvin *et al.*, 2010).

Although frequently cited as the second most common neurodegenerative dementia (McKeith *et al.*, 2017), there is little consensus regarding the prevalence of DLB. In this chapter we will discuss the different methodologies used to investigate DLB case frequency, exploring the strengths and limitations in each and discussing why comparison of the findings of different studies can be challenging.

2.2 DLB prevalence and incidence

The majority of studies investigating DLB prevalence and incidence are cross-sectional surveys of dementia populations and often adopt one of two main types of methodology in defining a DLB case. The first type of study defines DLB cases on the basis of Lewy body pathology detected in *post-mortem* samples (neuropathological studies); the second type on the basis of the *antemortem* clinical signs and symptoms characteristic of DLB (clinical studies). As well as comparing the findings from these two groups, this chapter will discuss the strengths and limitations of each.

Prior to discussing DLB prevalence and incidence, it is important to clearly define what we mean by these terms. Prevalence is the proportion of cases in population at a given point in time (Coggon, Rose and Barker, 2003), and in the case of DLB prevalence is discussed throughout previous studies as the percentage of cases with DLB within a dementia cohort (Vann Jones and O'Brien, 2014). Similarly, incidence is expressed as a percentage of new DLB cases among newly diagnosed dementia cases (Vann Jones and O'Brien, 2014). This chapter will therefore refer to prevalence and incidence in these terms.

Both prevalence and incidence are useful ways of discussing DLB frequency. All-cause dementia cases provide us with a conceptually and numerically stable denominator (Matthews *et al.*, 2016) with which clinicians are familiar and comfortable; as a numerator, DLB may be less well understood in clinical practice, having undergone conceptual evolution over the past two decades (McKeith *et al.*, 1996, 2005, 2017). Since DLB cases, when not recognised, are likely to be misdiagnosed as AD rather than a non-dementia diagnosis (Gaugler *et al.*, 2013; Galvin, 2015), referring to variation in detection or recognition as a percentage of all dementia cases is practical and can be easily contextualised to clinical services.

Incidence has been investigated in considerably fewer studies than DLB prevalence (Miech *et al.*, 2002; Matsui *et al.*, 2008; Perez *et al.*, 2010; Savica *et al.*, 2013) and for both this reason, and to enable comparison between neuropathological and clinical study designs, the discussion will focus on DLB prevalence.

2.2.1 DLB prevalence in neuropathological cohorts

Of the two methodologies described above, neuropathological studies typically report the higher rates of DLB prevalence, with most studies identifying pathology in 15-30% of subjects with dementia. In a hospital-based consecutive autopsy group comprising 1 100 subjects over the age of 70 with dementia, DLB was noted as the predominant pathology in 8.5%, with a further 8.9% demonstrating mixed AD and DLB pathology (Jellinger and Attems, 2011). One sample researching a community sample of 601 people aged 75 or over described DLB in 22% of 137 dementia cases (Rahkonen *et al.*, 2003) and the Vantaa 85+ study found that 36% of 304 subjects had pathological evidence of LB disease (Oinas *et al.*, 2009).

Interpretation of this data in a clinical context is challenging, as α-synuclein pathology has been found in up to 11% of patients without dementia at death (Wakisaka *et al.*, 2003) and in patients with other dementia subtypes (Ince, 2001; Jellinger, 2003). There is no consensus regarding a threshold of α-synuclein pathology that accurately differentiates patients with dementia from healthy older controls (Ince, 2001; Sonnen *et al.*, 2007; Zaccai *et al.*, 2015).

Most neuropathological studies therefore reflect the extent of pathology observed, rather than whether or not this was related to clinical expression of DLB symptoms. This is further complicated by the high proportion of DLB cases that have comorbid AD findings, and that in such cases the clinical phenotype may more closely resemble AD than DLB (Tiraboschi *et al.*, 2006; Weisman *et al.*, 2007; Thomas *et al.*, 2018b). There is therefore a strong possibility that a proportion of DLB cases, both pure and with comorbid AD pathology, will not and indeed could not be detected in routine clinical practice using current diagnostic methods.

Other factors further challenge clinical contextualisation of neuropathological data. Sample sizes in autopsy studies are generally smaller than in clinical studies and have been demonstrated to be subject to selection bias; subjects volunteering for autopsy studies are more likely to be white, married, more highly educated and have a longer duration of symptoms than those in a clinical population (Fillenbaum *et al.*, 1996; Tsuang *et al.*, 2006). Some studies, such as the Cognitive Functioning and Ageing Study (CFAS) (Ince, 2001) have attempted to recruit from community samples in an effort to minimise referral bias, but are still subject to consent bias, considerable attrition bias and a relative lack of ethnic variation (Zaccai, Ince and Brayne, 2006).

It is also difficult for autopsy studies to correlate clinical findings with neuropathological data. When classifying participants as dementia cases or healthy controls, retrospective studies rely on incomplete or unreliable data (Zaccai, Ince and Brayne, 2006). Some studies regularly collect *antemortem* data with standardised clinical tools (Ince, 2001; Polvikoski, Sulkava and Myllykangas, 2001), but a duration of up to two years between assessments mean that the cognitive status at last interview may not be representative of that immediately prior to death (Zaccai, Ince and Brayne, 2006).

In summary, neuropathological studies reflect the extent of pathology, but not the clinical expression of DLB symptoms, which may not be present in a proportion of cases. Factors such as selection bias and small sample sizes may contribute to the higher rates of DLB prevalence described in *post-mortem* studies. Neuropathological studies provide a useful upper margin for DLB prevalence but generalising their findings to clinical populations is difficult.

2.2.2 DLB prevalence in clinical cohorts

Clinical studies of DLB prevalence screen the entirety of an at-risk population (e.g. residents over 60 years old), or a random sample of a population in a particular community or region. Participants typically complete standardised screening tools before undergoing further assessment if dementia is suspected. DLB prevalence in these studies can often therefore expressed both as a proportion of all residents in the cohort studied and of dementia cases in the cohort studied (Stevens *et al.*, 2002; Rahkonen *et al.*, 2003; Yue *et al.*, 2016).

A 2014 meta-analysis of 18 such DLB prevalence studies, comprising a collective 26 137 patients aged 65 and over, reported that DLB represented 4.24% (95% CI 3.44–5.17%) of all dementia cases (Vann Jones and O'Brien, 2014). There was, however, considerable variation demonstrated among contributing studies; three studies, collectively comprising 3 579 cases, 290 patients of whom had dementia, failed to identify a single DLB case between them (Yamada *et al.*, 2002; Arslantaş *et al.*, 2009; Yusuf *et al.*, 2011). Although a Finnish study of 601 individuals over the age of 75 reported a DLB prevalence of 21.9% (Rahkonen *et al.*, 2003), diagnosis was made on the basis of *post-mortem* examination, and its presence as an outlier underlines that it more closely represents a neuropathological study than others contributing to meta-analysis; no other study included reported a DLB prevalence higher than 10% (Vann Jones and O'Brien, 2014).

Since publication of meta-analysis, the largest single clinical DLB prevalence study to date has been published, totalling 5 542 people in a rural Chinese population aged 60 and over and reporting that DLB cases represented 10.1% of 574 dementia cases (Yue *et al.*, 2016). Although this figure included patients likely to have PDD rather than DLB, previous studies adopting the same practice have reported similar prevalence rates (9.1-9.7%) (Stevens *et al.*, 2002; Gascón-Bayarri *et al.*, 2007; Fernández Martínez *et al.*, 2008). Among these three is the sole population-based prevalence study conducted in a UK population (Stevens *et al.*, 2002).

Clinical studies offer an advantage over neuropathological studies in helping to understand how commonly the clinical DLB phenotype can arise in communities. The systematic approach that they adopt in identifying symptoms and diagnoses can circumvent the referral bias that clinical studies may be subject to, potentially identifying dementia in the 43% of patients that do not present to services (Lang *et al.*, 2017). Although the diagnostic processes used in these studies are not as accurate as the gold standard of neuropathological diagnosis, some studies combine the two study designs, following up cases with *post-mortem* examination (Rahkonen *et al.*, 2003; Savica *et al.*, 2013).

Clinical epidemiological studies also have their limitations. The multi-tiered approach to case identification can, in some instances, mean that any screening process with sub-optimal sensitivity can lead to exclusion of large numbers of cases. This is demonstrated by a large DLB prevalence study which used recorded parkinsonism as a means of identifying the population at risk of DLB (Savica *et al.*, 2013). Not only did recruitment depend of on the sensitivity of the clinician identifying parkinsonism, but it excluded the 15-20% of patients with DLB that never demonstrate parkinsonism during the course of their illness (Harvey, Skelton-Robinson and Rossor, 2003; Ferman *et al.*, 2006; Walker *et al.*, 2016). The same study also illustrated a comparative weakness of population-based studies in comparison to neuropathological studies; although agreement between *antemortem* and *post-mortem* diagnosis was very high (94.1%), one of the 17 patients that underwent neuropathological examination demonstrated AD features with no evidence of LB disease (Savica *et al.*, 2013).

Another problem in interpreting DLB prevalence-based data is the small sample sizes included in such studies. Of 18 papers contributing to meta-analysis (Vann Jones and O'Brien, 2014) only three (Rahkonen *et al.*, 2003; Gascón-Bayarri *et al.*, 2007; Fernández Martínez *et al.*, 2008) identified more than ten DLB cases, and in all three of these studies, patients with PDD were included among DLB cases. The single largest all-cause dementia sample size included in meta-analysis is 351, lower than that used in many clinical prevalence studies (Vann Jones and O'Brien, 2014).

The clinical studies described thus far all define their at-risk populations on the basis of characteristics like age, and the majority of these participants would not have been in contact with

clinical services. However, a large proportion of clinical studies have investigated DLB prevalence in dementia populations seen within health services. For the purposes of this thesis, these studies of DLB prevalence are the most important to consider in greater detail.

When considering the real-life detection of DLB, and how it can be optimised, clinical prevalence in the context of cases presenting to secondary services therefore provides the most helpful reference point for discussion. It is a practically useful measure for several parties; for the researcher, in helping determine how many potential participants could be recruited from a particular service; for the manager, in determining the extent to which indicative biomarkers like FP-CIT SPECT and MIBG scintigraphy could be funded; and for the clinician, in helping benchmark his/her diagnostic sensitivity by comparing their own case detection rate with that reported elsewhere. It is around the characteristics of patients presenting to memory and cognitive services, rather than around cases in the wider community, that management pathways have been constructed (NICE, 2006). Were new methods of optimising DLB detection to emerge, such as new biomarkers, or validated assessment tools (Galvin, 2015; Thomas *et al.*, 2017b), clinical prevalence before and after the introduction of such methods can help quantify their clinical usefulness.

Numerous studies have investigated the prevalence of DLB in secondary care clinical populations. A 2014 meta-analysis (Vann Jones and O'Brien, 2014) included ten studies reporting the prevalence of DLB (Londos *et al.*, 2000; Chan *et al.*, 2002; Harvey, Skelton-Robinson and Rossor, 2003; Takada *et al.*, 2003; Sambrook *et al.*, 2004; Yokota *et al.*, 2005; Shinagawa *et al.*, 2007; Aarsland *et al.*, 2008; Alladi *et al.*, 2011; Yoshida *et al.*, 2011). These studies collectively totalled 3 144 participants with dementia and reported that DLB comprised 7.47% (95% CI 6.58–8.45) of this cohort. However, there was considerable variation in the frequency rates reported in individual studies; DLB comprised 2.18% (95% CI 0.80–4.69) of one cohort (Takada *et al.*, 2003) and 15.82% (95% CI 11.01–21.69) of another (Aarsland *et al.*, 2008).

Direct comparison of individual studies is challenged by a considerable variation in methodology. Only two of ten studies in secondary care populations (Aarsland *et al.*, 2008; Alladi *et al.*, 2011) contributing to this meta-analysis used the third consensus criteria (McKeith *et al.*, 2005), which has demonstrated higher sensitivity (85% (McKeith *et al.*, 2000b)) than the 1996 criteria (McKeith

et al., 1996) used in the remaining eight studies (75% (Mega *et al.*, 1996)). Both reported DLB frequency rates higher than the 7.47% mean generated by the meta-analysis.

Other characteristics also contributed to the heterogeneity of included studies. Small sample sizes (ranging from 102 to 766) resulted in wide confidence intervals in each cohort. Several were conducted predominantly in specialist or tertiary care services (Takada *et al.*, 2003; Sambrook *et al.*, 2004; Shinagawa *et al.*, 2007; Alladi *et al.*, 2011), inpatient populations (Chan *et al.*, 2002), or among younger patients with dementia (Harvey, Skelton-Robinson and Rossor, 2003; Shinagawa *et al.*, 2007) while others took place in more clinically representative settings. Differences between studies also go beyond recruitment; four of ten papers published did not include a neurological examination as part of their methodology (Londos *et al.*, 2000; Chan *et al.*, 2002; Sambrook *et al.*, 2004; Yoshida *et al.*, 2011), and each applied varying inclusion and exclusion criteria. Importantly, only two studies included a longitudinal component to their study design (Harvey, Skelton-Robinson and Rossor, 2003; Takada *et al.*, 2003; Sambrook *et al.*, 2004), thus failing to account for the emergence of core symptoms after initial diagnosis, and therefore the possibility of diagnostic revision.

Studies of clinical DLB prevalence published since this meta-analysis have reported case frequency rates lower than the 7.47% reported by Vann Jones and O'Brien (2014) (Avila-Castells *et al.*, 2012; Ikejima *et al.*, 2012; El Tallawy *et al.*, 2013; Garcia-Ptacek *et al.*, 2014; Sadak *et al.*, 2014; Goodman *et al.*, 2017) *(Table 2.1)*.

Among these was a study that included the single largest cohort of DLB patients in the literature to date. Goodman *et al.* (2017) reported the prevalence of dementia subtype diagnoses from 21.6 million patients over the age of 68 and enrolled in USA federal health insurance schemes (*Medicaid* and *Medicare*) between 2011 and 2013. The authors reported that DLB and PDD together comprised 5.4% (n= 168,629) of 3.1 million patients with dementia. However, the same study reports that 46.1% never received a subtype diagnosis other than *"Dementia (not otherwise specified)"* and *Medicaid* and *Medicare* data has been demonstrated to have poor sensitivity for subtype diagnosis when compared with structured assessment methods (Taylor, Jr. *et al.*, 2009). The study also investigated patients enrolled in the most basic of *Medicare* packages and

therefore likely to over-represent individuals from lower socio-economic groups. (Goodman *et al.*, 2017)

Table 2.1 DLB prevalence studies in secondary and tertiary care populations

Study	Year	DLB (n)	Dementia (n)	DLB Prevalence % (95% CI)
Londos *et al.**	2000	48	200	24.0 (18.3 – 30.5)
Chan *et al.*	2002	3	102	2.9 (0.6 – 8.4)
Harvey *et al.*	2003	12	185	6.5 (3.4 – 11.1)
Takada *et al.*	2003	6	275	2.2 (0.8 – 4.7)
Sambrook *et al.**	2004	23	766	3.0 (1.9 – 4.5)
Yokota *et al.*	2005	17	464	4.0 (2.3 – 6.3)
Shinagawa *et al.*	2007	53	483	11.0 (8.3 – 14.1)
Aarsland *et al.*	2008	39	196	15.8 (11.0 – 21.7)
Yoshida *et al.*	2011	11	126	8.7 (4.4 - 15.1)
Alladi *et al.*	2011	31	347	8.9 (6.2 – 12.4)
Avila-Castells *et al.**	2012	100	1 894	5.3 (4.4 - 6.4)
Ikejima *et al.*	2012	35	768	4.6 (3.3 – 6.3)
Bonanni et al.	2013	2 042	541	26.5 (24.6 – 28.5)
El Tallawy et al.*	2013	7	87	8.1 (4.0 – 15.7)
Garcia-Ptacek *et al.*	2014	461	15 209	3.0 (2.8 – 3.3)
Sadak *et al.*	2014	241	3 768	6.4 (5.7 – 7.2)
Goodman *et al.*	2017	168 629	3 110 654	5.4 (5.4 – 5.5)

**Reported prevalence of DLB and PDD combined*

Another large DLB prevalence study, (Bonanni *et al.*, 2013), conducted among a dementia population of 2 042 Italian participants, reports that DLB comprises 25-28% of new dementia cases in a range of secondary and tertiary services. When considered alongside neuropathological prevalence studies, this figure would appear unfeasibly high, particularly in a clinically representative population. This study is discussed further in Chapter *6.2.2.*

One of the difficulties presented by the heterogeneity of both clinical and neuropathological studies to date is the failure to consider possible variation in the true prevalence of DLB between

different countries, and thus the possibility that environmental factors may precipitate or predispose certain populations to DLB. Among countries represented in populations discussed above, only Japan contributed more than one study, and variation in DLB case frequency (3.95 - 10.97%) and differences in study methodology, even between these three studies, precludes comparison between these populations (Shinagawa *et al.*, 2007; Yokota *et al.*, 2007; Yoshida *et al.*, 2011).

Similarly, studies comparing several services in the same region might help determine whether the practice or expertise of clinicians in each service, rather than environmental factors within the region, might contribute to variation in DLB prevalence. However, only one study (Bonanni *et al.*, 2013) has compared DLB prevalence between different services and identified no significant differences in the proportion of DLB observed in secondary and tertiary care dementia services.

The same factors that contribute to variation between studies also factors limit generalisability of individual studies, and meta-analysis, to a UK population. A single study contributing to meta-analysis was conducted in the UK (Harvey, Skelton-Robinson and Rossor, 2003), and itself investigated dementia subtypes in younger onset dementia patients. Furthermore, neither this study, nor any of those included in meta-analysis, reflect populations representative of those seen in UK populations, recruiting from neurology, geriatric medicine or neuropsychiatry services; in contrast the majority of dementia cases in UK clinical practice are diagnosed by an Old Age Psychiatrist.

The difficulties in comparing individual DLB prevalence studies, and in generalising this body of evidence to a UK clinical population, also present challenges in understanding the relationships between age, gender and DLB prevalence, particularly as several studies have failed to report such characteristics of their samples. Vann Jones and O'Brien (2014) reported that of 28 prevalence samples contributing to their meta-analysis, eight DLB samples reported gender composition. Of these eight studies, five observed a preponderance of female patients in their sample population. PDD is recognised as being more common in men (Mayeux *et al.*, 1995) and recent large-scale DLB studies have reported significant higher rates of male patients in their samples (Savica *et al.*, 2013; Goodman *et al.*, 2017), although others have failed to identify any preponderance (Yue *et al.*, 2016).

The ages of DLB samples are more consistently reported than gender, but considerable variation is observed in prevalence and incidence studies; of those contributing to meta-analysis (Vann Jones and O'Brien, 2014) mean ages ranged from 58.7 years (Harvey, Skelton-Robinson and Rossor, 2003) to 79.0 years (Chan *et al.*, 2002). The only study of subtype prevalence in dementia populations under the age of 65 reported a comparatively high case frequency (6.5%) that might suggest a preponderance in younger populations (Harvey, Skelton-Robinson and Rossor, 2003) but larger samples have demonstrated consistent rates of DLB prevalence across the age groups of clinical samples (Yue *et al.*, 2016; Goodman *et al.*, 2017).

In summary, studies investigating DLB case frequency in clinical populations provide a useful means of understanding and discussing prevalence of the disease. Like population-based studies they identify cases on the basis of clinical phenotype, but generally do so using larger sample sizes by sampling directly from dementia populations. Although neuropathological studies offer gold standard accuracy, clinical prevalence is the most helpful measure in understanding DLB diagnosis in clinical services.

Despite this, more research in required into clinical DLB epidemiology, as existing studies have included small sample sizes, failed to include representative populations and failed to offer an insight into whether true disease prevalence may vary between different services and different regions. These studies have also failed to establish consistent relationships between DLB and both patient gender and age. These factors challenge the generalisation of current epidemiological research to UK practice, and underline the importance of a large-scale study of DLB prevalence in representative NHS services.

2.3 Understanding DLB recognition in clinical services

Understanding the detection of DLB in clinical services must go beyond examining the frequency of DLB among all-case dementia cases. Most epidemiological prevalence and incidence studies fail to capture the longitudinal nature of patients' contact with services; clinical diagnoses in these are largely based on single assessments (Chan *et al.*, 2002; Yokota *et al.*, 2005; Shinagawa *et al.*, 2007; Aarsland *et al.*, 2008; Alladi *et al.*, 2011; Yoshida *et al.*, 2011). Even when routinely followed up (Rahkonen *et al.*, 2003), assessment takes the form of structured interview and does not reflect the shorter, less focused nature of clinical follow-up.

One of the reasons why this is significant is that a proportion of patients with DLB are initially assigned an alternative dementia subtype diagnosis. Several clinicopathological studies described diagnostic revision at post-mortem examination, but as previously discussed (Chapter 2.2.1), extrapolating neuropathological findings to a clinical population can be challenging, as some cases autopsy-confirmed DLB pathology will not demonstrate detectable clinical features *antemortem*, particularly if comorbid AD pathology is present. A more suitable group of studies with which to compare naturalistic diagnostic revision are those that incorporate longitudinal assessment or review of clinical notes.

Three of twenty-five cases (12%) recruited to one such autopsy study were diagnosed with AD at baseline, but developed core features later in their presentation; all three demonstrated DLB pathology at *post-mortem* examination (Thomas *et al.*, 2017a). The same group also reported on 22 neuropathologically mixed DLB/AD cases, of whom 14 were given an initial diagnosis of AD (Thomas *et al.*, 2018b). Seven of these clinical AD cases later developed features of DLB and either given a subsequent diagnosis of DLB (n=2) or mixed DLB/AD (n=5). These findings are supported by data from a cross-sectional survey conducted by the USA Lewy Body Dementia Association among 962 carers of patients with either DLB or PDD, reporting that of patients presenting to clinicians with cognitive symptoms, 19% were initially diagnosed with AD, 5% with FTD, 4% VaD and 4% unspecified dementia (Galvin, 2015).

One of the reasons that revision of diagnosis may have occurred in these three studies was that opportunities were identified to reappraise diagnosis on the basis of emergence of new clinical features, either through study design (Thomas *et al.*, 2017a; Thomas *et al.*, 2018b), or by routine clinical care (Galvin *et al.*, 2010). The latter provides data on the extent of contact with services, reporting that patients attended a mean of 3.7 ± 1.9 appointments before DLB or PDD diagnosis was made, and more than six visits were required in 33% of cases. This observation was accompanied by variation in time to diagnosis; 19% percent of respondents were diagnosed within a month of first contact with services and 51% were diagnosed within one year (Galvin *et al.*, 2010).

The differences in USA and UK dementia healthcare (Knapp *et al.*, 2007) prevent generalisation of this caregiver survey to NHS practice, and as a retrospective survey, findings may be subject to

recall bias. Although delays in diagnosis have been studied in NHS populations, these have focused primarily on delay in referral to specialist services on the part of general practitioners (GPs), and have examined the dementia syndrome rather than subtype diagnosis (O'Connor *et al.*, 1988; Bamford *et al.*, 2007; Koch, Iliffe and project, 2010).

Any helpful discussion of how DLB is recognised and diagnosed in clinical services should therefore include consideration of diagnostic revision and investigate the opportunities that arise during routine clinical care to reappraise clinical symptoms and subtype diagnosis.

2.4 Summary

Despite the importance of DLB recognition in guiding clinical management and providing accurate information to patients and their carers, there is a lack of consensus regarding the frequency of DLB in dementia services. Although two broad groups of study design have been employed in attempting to rectify this, each has their respective strengths and limitations and report DLB as representing varying proportions of dementia cases.

Clinical prevalence studies offer findings most relevant to understanding the prevalence of DLB in routine clinical care, but studies of this type have reported wide variation in methodology and primary findings, limiting our ability to compare studies and impairing their generalisability to the NHS services. An investigation into the clinical prevalence of DLB in a UK population, preferably involving several discrete services, is therefore important.

Further understanding DLB detection requires an investigation into how frequently cases are initially assigned a subtype diagnosis other than DLB, as this has been reported in some studies with longitudinal data collection. Examining the characteristics of DLB patients' contact with services, including the frequency of opportunities to reappraise diagnosis, and the duration of time over which symptoms evolve in order to prompt diagnostic reappraisal, would further improve our understanding of detection in the clinical environment. Chapter *4* describes such a study undertaken within UK clinical services.

Chapter 3

Imaging in DLB diagnosis

3.1 Introduction

Chapter 2 described the difficulties in interpreting the varied and limited evidence base surrounding DLB epidemiology but concluded that DLB nevertheless appears to comprise a substantial proportion of cases of neurodegenerative dementia. The chapter also discussed the importance of accurate DLB diagnosis (Hanyu *et al.*, 2009; Lee *et al.*, 2013; Stinton *et al.*, 2015), but noted that many cases are not recognised in the clinical setting, and are often misdiagnosed as AD.

The development of suitable biomarkers may provide a mechanism by which DLB case detection could be enhanced. Successive DLB diagnostic criteria have incorporated biomarkers capable of distinguishing DLB from AD. The third consensus report included striatal impairment detected by FP-CIT SPECT as a suggestive feature for DLB, and the fourth consensus criteria listed FP-CIT and MIBG cardiac scintigraphy as indicative biomarkers (McKeith *et al.*, 2017).

This chapter will provide a brief overview of imaging methods used in DLB research and clinical diagnosis, focusing on these two indicative biomarkers.

3.2 Structural imaging

CT and MRI are methods of assessing structural changes to the brain related to pathology and are capable of identifying the presence of cerebrovascular disease and reversible aetiologies manifesting as dementia, such as normal pressure hydrocephalus and neoplasms. Brain CT or MRI is therefore recommended by UK national guidelines for every patient presenting to memory services (NICE, 2006). MRI provides superior contrast and specific tissue characterisation to CT, which is cheaper, more widely available and can now be done very rapidly (Watson and Colloby, 2016).

Structural imaging methods, particularly MRI, have demonstrated some capability in differentiating DLB from AD. In particular, preservation of the MTL is sensitive (91%) and specific (94%) in distinguishing DLB from AD (Burton *et al.*, 2009) and has been included as a supportive

biomarker in the fourth consensus criteria (McKeith *et al.*, 2017). Interpretation of MTL atrophy is complicated, however, by the high prevalence of concurrent AD pathology in DLB cases (Jellinger and Attems, 2008); the rates of atrophy seen in patients with mixed DLB/AD are similar to those observed in subjects with AD pathology alone (Nedelska *et al.*, 2015). While the absence of MTL atrophy in the presence of a degenerative dementia is therefore suggestive of DLB, the presence of MTL atrophy is consistent with mixed AD and LB pathology as well as an absence of LB pathology.

3.3 Nuclear Medicine

In spite of their higher cost, the functional imaging methods, FP-CIT and MIBG cardiac scintigraphy, carry greater diagnostic weight in consensus criteria than MTL preservation on CT or MRI (McKeith *et al.*, 2017). The fourth consensus criteria also include two other supportive functional imaging biomarkers; low perfusion on HMPAO SPECT and reduced metabolism on FDG PET.

The nuclear medicine techniques of PET and SPECT allow observation and measurement of some aspects more closely associated with synaptic dysfunction, rather than frank neuronal loss alone. Both techniques involve visualisation of functional analogues via measurement of emissions from attached radioisotopes. These are labelled with a radioactive moiety, most usually iodine or technetium (for SPECT) or carbon or fluorine (for PET) to tracers, after which they are administered to the patient via intravenous injection. Both PET and SPECT measure the emission of γ-rays produced from the decay of the radioisotope; the difference between the two modalities is the mechanism by which these γ-rays are generated.

In PET imaging, the positron emitted following the decay of the isotope is mutually annihilated with an electron from the surrounding area, producing two 511keV γ-rays travelling in opposite directions. Opposing pairs of scintillation detectors within PET scanners simultaneously register these pair of photons (the "*coincidence event*") and enable tracing of their lines of response (LOR). Data derived from both the coincidence event and LOR are used to determine the source of positron annihilation, and thus tracer location, and are subsequently converted into a tomographic image.

SPECT imaging differs from PET in that γ-rays are emitted in the form of single photons, rather

than produced through annihilation. Both modalities offer high detection sensitivity and good spatial resolution, and are capable of producing tomographic images, but PET provides higher localisation and image resolution than SPECT (Lu and Yuan, 2015). PET radiopharmaceuticals generally have a shorter half-life than those used in SPECT imaging, in some cases requiring the proximity of a cyclotron for manufacture and can therefore be more expensive (Lu and Yuan, 2015).

Nuclear medicine investigations come with important considerations that may limit their routine clinical use. Acute side effects of nuclear medicine techniques are rare, with 0.9 events per 100 000 investigations reported (Matsuda *et al.*, 2016). Although radiation exposure from radiopharmaceuticals are associated with an increased risk of malignancy (Soucy *et al.*, 2013), the dose of radiation from FP-CIT SPECT, for example, is equivalent to exposure from background radiation in the UK over two years (Public Health England, 2018). Prophylactic potassium iodide can decrease thyroid dose from iodine-labelled radiopharmaceuticals (Nauman and Wolff, 1993), but this it brings its own risks of allergic reactions and gastrointestinal disturbance; however, these instances are believed to be very rare (Sicherer, 2004).

Nuclear Medicine methods can be more expensive than structural imaging investigations such as CT and MRI (Rayment *et al.*, 2016), by virtue not only of the cost of equipment, materials, and expertise, but in many cases by the additional human resource requirements associated with the presence of the patient and carer at facilities for several hours prior to image acquisition. This longer duration may be compounded by travel time to and from the specialist facilities, and complicated by clinical characteristics associated with an elderly population, such as cognitive impairment, mobility symptoms, or frailty.

3.3.1 FDG PET

The uptake of FDG, a glucose analogue, is used as a measure of glucose metabolism in PET imaging, highlighting areas of hypometabolism consistent with areas of dysfunction and neurodegeneration suggestive of dementia. Patients with DLB and AD demonstrate overlapping but distinct patterns of regional hypometabolism (Watson and Colloby, 2016). In AD, hypometabolism is reported as commonly affecting temporoparietal areas, with the posterior cingulate and medial temporal areas particularly affected, and sensory motor cortices largely

spared. In DLB, reductions in occipital activity are frequently observed, with significant reduction in the primary visual cortex demonstrating 90% sensitivity and 80% specificity for differentiating DLB from AD in one autopsy-confirmed sample (Minoshima *et al.*, 2001). However, larger studies, with scans conducted earlier in the course of disease, have observed a lower sensitivity (67-70%) and specificity (74-92%) for occipital hypometabolism in differentiating DLB from AD (Ishii *et al.*, 1998; O'Brien *et al.*, 2014a).

The "*cingulate island sign*" - the relative preservation of the posterior cingulate relative to precuneus and cuneus, has also been suggested as a characteristic FDG PET finding in DLB, demonstrating superior sensitivity (83%) and specificity (93%) than occipital hypometabolism for discriminating DLB and AD (Lim *et al.*, 2009), although these findings have yet to be supported by a multicentre study.

In summary, FDG PET shows promise as a biomarker in differentiating DLB from AD but comparatively small sample sizes and the inconsistency in reported utility are reflected in its suggestive, rather than indicative, biomarker status (Watson and Colloby, 2016; McKeith *et al.*, 2017; Surendranathan and O'Brien, 2018).

3.3.2 HMPAO SPECT

^{99m}Tc-hexamethyl propyleneamine oxime (HMPAO) SPECT uses regional cerebral blood flow, rather than regional metabolism, as a means of measuring neuronal activity. As a consequence, HMPAO SPECT demonstrates uptake patterns similar to those observed in FDG PET studies; occipital and temporal lobe hypoperfusion are used as markers for DLB and AD respectively (Watson and Colloby, 2016).

Studies assessing the clinical utility of HMPAO SPECT showed variable DLB sensitivity (64%-85%) and AD specificity (64%-87%) but were characterised by small sample sizes (Yeo *et al.*, 2013). Although HMPAO had initially been favoured over FDG PET due to its wider availability, lower cost and perceived better patient tolerability, the superiority of the former in both discriminating DLB from AD, and degenerative dementia from healthy controls, has been demonstrated, and a marked decrease in the cost of FDG PET in recent years clearly supports its use over HMPAO (O'Brien *et al.*, 2014a).

3.3.3 Amyloid PET

The development of amyloid radiopharmaceuticals, such as ^{11}C Pittsburgh compound B, which is taken up by both diffuse and neuritic plaques, has allowed *in vivo* measurement of amyloid pathology, previously confined to *post-mortem* examination alone. Amyloid PET has been studied extensively in AD populations and incorporated into revised AD criteria (McKhann *et al.*, 2011), but it is less well established in DLB populations. Amyloid binding is higher in DLB than PDD and healthy controls, compared to in AD (Donaghy, Thomas and O'Brien, 2015) but the amyloid PET negativity in up to half of DLB patients, and the prevalence of amyloid positivity in healthy controls (Ossenkoppele *et al.*, 2015), limit its use as a diagnostic tool. Use has generally been limited to research studies and amyloid PET is not included in the fourth DLB consensus report as an indicative or suggestive biomarker for DLB (McKeith *et al.*, 2017).

3.3.4 FP-CIT SPECT

^{123}I-FP-CIT SPECT (FP-CIT) assesses the presence of nigrostriatal degeneration, by visualising radiopharmaceutical uptake at the dopamine transporter reuptake sites in the striatum. As a reduction in dopamine terminals is observed in the striata of patients with DLB, subjects demonstrate significantly reduced striatal FP-CIT uptake compared with both their AD counterparts and healthy older controls (Walker *et al.*, 2002; O'Brien *et al.*, 2004).

Studies using both *ante-mortem* clinical diagnosis and *post-mortem* neuropathological findings as gold standard measurements have supported the utility of FP-CIT as a biomarker, consistently reporting high specificity, if variable sensitivity, for DLB against other dementia subtypes. An early study investigating an autopsy-confirmed cohort reported a DLB sensitivity of 88% and a non-DLB specificity of 100% (Walker *et al.*, 2007). A larger study comparing FP-CIT with clinical diagnosis observed a DLB sensitivity of 78% and AD specificity of 88% (O'Brien *et al.*, 2004). The largest single FP-CIT study to date, a multicentre trial comprising 147 patients with probable or possible DLB, reported a mean sensitivity and non-DLB specificity of 78% and 90% respectively (McKeith *et al.*, 2007), again comparing FP-CIT against the clinical diagnosis.

A recent study compared *ante-mortem* clinical diagnosis with FP-CIT in a cohort of 30 pure DLB or mixed DLB/AD cases (Thomas *et al.*, 2017a). FP-CIT demonstrated an 80% DLB sensitivity and 92% AD specificity, compared with the 87% sensitivity and 72% specificity of clinical assessment. The

superior overall accuracy of FP-CIT in this study (86%) compared to clinical diagnosis (79%) supports a previous meta-analysis reporting similar observations (Brigo, Turri and Tinazzi, 2015).

A number of other factors beyond the clinical utility of FP-CIT positively influence its validity as a biomarker. Procedural guidelines for the method have been established and it is reproducible both within and between different research centres (Darcourt *et al.*, 2010; Seibyl *et al.*, 2014). Factors like age and gender on FP-CIT positivity have been investigated (Varrone *et al.*, 2013), and procedures for interpretation are well established (Benamer *et al.*, 2000). Low dopamine transporter uptake was therefore included as a suggestive DLB feature in the third (McKeith *et al.*, 2005) and an indicative biomarker in the fourth DLB consensus criteria (McKeith *et al.*, 2017).

A further practical consideration in FP-CIT is the absence of evidence to suggest significant interactions between the radiopharmaceutical and prescribed medications. Although medications that bind to the dopamine transporter with high affinity have been hypothesised to interfere with FP-CIT uptake and interpretation (Darcourt *et al.*, 2010), only modafinil has been implicated as doing so in a published human study (Borghammer *et al.*, 2014), and only amphetamines have been recommended to clinicians as appropriate to stop prior to FP-CIT (Booij and Kemp, 2008).

There are, however, important limitations to the use of FP-CIT. Despite its excellent reported specificity, values for sensitivity are consistently reported in the moderate range (78%-80%)(O'Brien *et al.*, 2004; McKeith *et al.*, 2007; Thomas *et al.*, 2017a). Thomas *et al*. (2017a) reported a higher specificity and overall accuracy of FP-CIT over clinical diagnosis, but a higher sensitivity was observed in the latter (87% vs 80%). The same study reported negative FP-CIT findings in three cases among their neuropathologically-confirmed cohort of 30 DLB patients. Each of these cases demonstrated predominantly neocortical or limbic pathology rather than striatal disease, and had presented with fluctuations and visual hallucinations, suggesting that FP-CIT may have limited sensitivity in such clinical groups.

A further three cases from this study met clinical criteria for probable AD at the time of scanning, but subsequently developed DLB symptoms, indicating that striatal impairment in some cases may not be sufficient to produce an abnormal FP-CIT result in the early stages of disease (Thomas *et al.*, 2017a). This has been supported by a study from the same group, reporting that FP-CIT

demonstrated limited sensitivity (54.2%) but good specificity (89.0%) in distinguishing MCI with LB disease from MCI in AD (Thomas *et al.*, 2018a).

This absence of substantial striatal pathology in a proportion of patients with neocortical Lewy body disease is supported by findings in community-based neuropathological cohorts (Zaccai *et al.*, 2015) and raises the theoretical, though currently unsubstantiated, possibility that FP-CIT sensitivity may be lower in certain DLB disease phenotypes; in particular, patients without parkinsonian symptoms. Since the dopamine transporter loss measured by FP-CIT has been shown to be less pronounced in subjects without parkinsonism (Piggott *et al.*, 1999), and since less than 85% of DLB patients demonstrate such symptoms during the course of their illness (Harvey, Skelton-Robinson and Rossor, 2003; Ferman *et al.*, 2006; Savica *et al.*, 2013; Walker *et al.*, 2016), it might be expected that FP-CIT produces a higher rate of false negative results in those without extrapyramidal features than in other DLB phenotypes. FP-CIT utility has not been researched specifically in patients without parkinsonism, but the absence of striatal pathology in neuropathologically confirmed DLB cases, albeit in small sample sizes, could encourage investigation of alternative biomarkers in such groups.

As well as the potential for these false negative FP-CIT findings, clinically relevant false positive results can also occur. Frontotemporal dementia, basal ganglia infarcts, progressive supranuclear palsy (PSP) and MSA, all of which can share clinical characteristics with DLB and PDD, can all produce positive FP-CIT findings (Zijlmans *et al.*, 2007; Vlaar *et al.*, 2008; Morgan *et al.*, 2012).

In summary, FP-CIT is validated as a biomarker for differentiating DLB from AD and non-DLB dementias, demonstrating excellent specificity and overall accuracy as reported by a range of studies. It is not, however, without its weaknesses, including a suboptimal sensitivity that may be related to certain disease phenotypes, and alternative biomarkers may be helpful in such populations.

3.4 MIBG cardiac scintigraphy

3.4.1 MIBG studies in clinical populations

^{123}I-metaiodobenzylguanidine (MIBG) is a radiopharmaceutical which permits visualisation and semiquantification of sympathetic innervation with planar scintigraphy or SPECT imaging. An

analogue of guanethidine, MIBG is taken up by the postganglionic presynaptic nerve endings, and radioactive labelling of MIBG permits visualisation of innervation *in vivo*. Semi-quantification of cardiac MIBG uptake, and thus degree of sympathetic denervation, is achieved through calculation of the heart to mediastinum ratio (HMR) of MIBG uptake on planar imaging. These images are acquired at 20 minutes ("*early*" images) and 240 minutes ("*late*" images) following radioisotope injection. A region of interest (ROI) is drawn over the heart, and a ROI positioned on the upper mediastinum. HMRs are calculated as a fraction of the mean count per pixel in the heart ROI divided by that in the upper mediastinum ROI.

First used to determine sympathetic dysfunction in congestive cardiac failure and myocardial infarction (MI) (Henderson *et al.*, 1988; Schofer *et al.*, 1988), MIBG was later employed to investigate systemic autonomic dysfunction in PD and came to be proposed as a means of distinguishing PD from MSA (Braune *et al.*, 1998; Orimo *et al.*, 1999). Watanabe *et al.* (2001). Yoshita *et al*. (2001) first reported significant differences in HMR uptake between DLB and AD patients.

Early work investigating MIBG in DLB populations was conducted among Japanese patients; six of the eight studies contributing to a 2012 meta-analysis of MIBG utility investigated Japanese cohorts (Treglia and Cason, 2012). Between 2001 and 2009, several studies observed MIBG's 100% sensitivity and specificity in differentiating DLB from AD (Watanabe *et al.*, 2001; Yoshita, Taki and Yamada, 2001; Oide *et al.*, 2003; Yoshita *et al.*, 2006; Noguchi-Shinohara *et al.*, 2009). Hanyu *et al*. (2006) described not only a 100% sensitivity and 92% specificity of MIBG for distinguishing DLB from AD, but also observed a lower MIBG uptake in patients with DLB than those with VaD, Parkinson's Plus dementia syndromes and FTD. MIBG had a 95% DLB sensitivity and 87% non-DLB specificity.

Yoshita *et al*. (2015) published the findings of their study of MIBG in the largest single DLB cohort to date (n=87; probable DLB n=61, probable AD n=46). Data was collected at ten Japanese sites, necessitating the development of a calibration method to standardise practice across participating centres (Nakajima *et al.*, 2012). An overall MIBG sensitivity of 69% and specificity of 89% to differentiate probable DLB from probable AD was reported, with higher accuracy reported (77% sensitivity, and 94% specificity) in patients with mild dementia (MMSE ≥ 22).

The favourable utility of MIBG in DLB diagnosis in these samples has contributed to the widespread use of MIBG in Japanese clinical practice (Nakajima *et al.*, 2008) and recognition of the modality as a supportive imaging tool by the nation's social health insurance authorities (Orimo *et al.*, 2016).

Four prospective European studies have investigated MIBG in DLB populations; three in Italian groups, and one in a Spanish cohort (Estorch *et al.*, 2008; Novellino *et al.*, 2010; Treglia *et al.*, 2012; Tiraboschi *et al.*, 2016). Estorch *et al*. (2008) reported on MIBG findings from a group comprising 19 Spanish DLB subjects (each with two or more clinical DLB features), as well as 12 AD patients and 13 recruits with other neurodegenerative diseases with cognitive impairment. A *post hoc* HMR cut-off of 1.36 corresponded to a sensitivity and specificity of MIBG of 94% and 96% respectively. Novellino *et al*. (2010) reported an abnormal HMR in all DLB patients (probable DLB n =8, possible DLB n=1) and normal HMR in FTD (n=6) and controls (n=16), with MIBG demonstrating a 100% accuracy in distinguishing the two groups.

3.4.2 MIBG and potentially interfering medications

Although the evidence base relating to MIBG has encouraging implications for clinical practice, the important limitations of the research conducted thus far make translation of research data into routine use difficult.

The majority of MIBG studies to date have excluded patients prescribed any medications suspected to interfere with MIBG uptake (Oide *et al.*, 2003; Hanyu *et al.*, 2006; Yoshita *et al.*, 2006; Wada-Isoe *et al.*, 2007; Estorch *et al.*, 2008; Noguchi-Shinohara *et al.*, 2009; Novellino *et al.*, 2010), citing guidelines by the European Association of Nuclear Medicine (Giammarile *et al.*, 2008), or a review relating to MIBG use in the treatment of neuroblastoma (Solanki *et al.*, 1992). As an analogue of noradrenaline, MIBG is transported into presynaptic terminals through a number of mechanisms; chiefly, the energy-dependent "*Uptake-one*" process mediated by the norepinephrine transporter (NET), a transmembrane protein (Streby *et al.*, 2015). Other processes also influence noradrenaline transport across the cell membrane, including active transport into vesicles, granular uptake, and calcium channel-mediated reuptake. Disruption of any of these mechanisms has the theoretical potential to diminish noradrenaline, and therefore MIBG, uptake (Giammarile *et al.*, 2008).

This practice of excluding patients on such medications, while important in accounting for confounding variables in an emerging evidence base, presents considerable difficulty in establishing MIBG in routine clinical practice. Among the medications identified as potentially interfering with MIBG are a number of commonly prescribed, clinically important agents that include analgesics, antidepressants, antipsychotics, cardiovascular medications and inhaled sympathomimetics used in the treatment of asthma and chronic obstructive pulmonary disease.

Marquié Sayagués *et al*. (2010) demonstrated the scale of exclusion of such patients in a 2010 non-controlled study investigating MIBG use in the diagnosis of DLB and PDD. Thirty one of the 77 patients (40%) recruited to the study were prescribed medications identified as potentially interfering with uptake; these included antipsychotics (n=26), levodopa (n=29; 38%), venlafaxine (n=4; 5%), and amitriptyline (n=2; 3%). Twenty-seven participants (35%) were also prescribed antihypertensive medication. The study failed to identify a significant difference in HMR between patients prescribed potentially interfering medications (HMR 1.47 ± 0.23) and those that were not (HMR 1.40 ± 0.33). That such a high proportion of participants were prescribed such medications in this clinically representative sample indicates the impracticability of restricting MIBG use in these patients, both in research and clinical settings.

The argument against excluding patients prescribed medications identified as interfering was strengthened further by a comprehensive review, which assessed published data on drug interactions with MIBG uptake (Jacobson and Travin, 2015). The authors noted that the majority of studies involved *in vitro* or non-human methodologies and recommended that only labetalol or tricyclic antidepressants should be withdrawn prior to MIBG imaging.

More recent MIBG utility studies have created inclusion and exclusion criteria with the intent of recruiting more clinically representative populations. Yoshita *et al*'s (2015) multicentre study excluded patients on the basis of reserpine or tricyclic antidepressants alone, while an Italian MIBG study withheld medications, rather than exclude their participants, prior to cardiac scintigraphy (Tiraboschi *et al.*, 2016). Withholding medications poses its own practical difficulties; restricting use of medications such as inhalers, analgesics or dopaminergic agents for even short periods could lead to an escalation in symptoms and deterioration in wellbeing.

3.4.3 MIBG and comorbidities

Similar concerns exist surrounding the exclusion of patients with medical comorbidities from MIBG utility studies. In determining the presence of impaired sympathetic uptake, MIBG studies in DLB diagnosis have presumed such findings to be attributable to neuronal denervation secondary to α-synuclein deposition; however, several common conditions are capable of producing similar findings. Early MIBG studies restricted the recruitment of patients with comorbidities. In order to prevent the possibility of reduced MIBG uptake in diabetic neuropathy, studies excluded patients with any history of diabetes mellitus (Hanyu *et al.*, 2006; Yoshita *et al.*, 2006; Wada-Isoe *et al.*, 2007; Estorch *et al.*, 2008; Noguchi-Shinohara *et al.*, 2009). Patients with "*heart disease*" were excluded to account for the generalised reduction in noradrenergic uptake observed in left ventricular dysfunction and the more localised reduction secondary to both chronic and acute myocardial ischaemia (Hanyu *et al.*, 2006; Yoshita *et al.*, 2006; Estorch *et al.*, 2008; Noguchi-Shinohara *et al.*, 2009; Novellino *et al.*, 2010; Wada-Isoe *et al.*, 2012). Subjects with thyroid disease, capable of producing autonomic dysfunction, and therefore impaired MIBG uptake, were excluded in two of these studies (Hanyu *et al.*, 2006; Yoshita *et al.*, 2006).

Marquié Sayagués *et al*. (2010) demonstrated that medical comorbidities affected a substantial proportion of the population likely to benefit from MIBG and found no significant relationship between HMR and comorbidity status. Twenty-four of 77 subjects had a history of "*diseases that may interfere with uptake*", including arrhythmias, MI and cardiac failure.

Two more recent studies have attempted to address the difficulty in translating the findings of early MIBG studies into clinical practice. Yoshita *et al*'s (2015) multicentre study did recruit a population with some comorbidities, including subjects with a history of ischaemic heart disease or myocardial blood flow abnormalities, provided they occurred more than six or twelve months respectively before consent. The same study did, however, also exclude patients with thyroid disease and insulin-controlled diabetes.

Tiraboschi *et al*. (2016) did not cite any medical illnesses in the exclusion criteria of their study, explicitly stating that they deliberately included subjects "*with common illnesses in the elderly (including ischemic, hypertensive, dilated cardiomyopathy, and diabetes) that might reduce ^{123}I-MIBG uptake*", in an effort to investigate a cohort representative of clinical practice. While the

same paper failed to cite how many of its participants were affected by such illnesses, it did report that DLB and non-DLB subjects reported a mean of 0.8 and 0.5 conditions (as structured by the Cumulative Illness Rating scale) respectively.

To summarise, although early MIBG studies excluded large proportions of patients on the basis of both medical comorbidities and prescribed medications, the high prevalence of these factors severely restricts the translation of such findings into clinical practice (Marquié Sayagués *et al.*, 2010). Although two more recent studies, conducted in Japan and Italy, have recruited more representative cohorts, the former reported the lowest overall accuracy of MIBG published to date, and the latter temporarily withdrew medications deemed capable of interacting with MIBG uptake (Yoshita *et al.*, 2015; Tiraboschi *et al.*, 2016).

3.4.4 Comparing MIBG and FP-CIT

As two of the indicative biomarkers listed by the fourth consensus criteria, direct comparison of FP-CIT and MIBG is important in guiding clinical decision making. Although as described above, some studies have shown sensitivity (98%) and specificity (94%) for MIBG superior to that of FP-CIT (sensitivity 79%, specificity 90%) (McKeith *et al.*, 2007; Treglia *et al.*, 2012), research relating to the latter has been conducted on more clinically representative populations.

Two Italian studies directly compare MIBG and FP-CIT in a concurrent group of patients (Treglia *et al.*, 2012; Tiraboschi *et al.*, 2016). Treglia *et al*. (2012) described four different combinations of FP-CIT and MIBG results in a group of 31 patients with dementia; 17 of 20 DLB patients (85%) had abnormal findings in both imaging modalities. Identical results for DLB sensitivity (90%), AD specificity and overall accuracy (91%) were reported for both FP-CIT and MIBG in this group.

Tiraboschi *et al*'s (2016) findings also described all four combinations of FP-CIT and MIBG result in a cohort comprising 30 DLB patients (probable DLB n=27, possible DLB n=3) and 29 recruits with a non-DLB dementia diagnosis. MIBG was reported to have a superior sensitivity (93%) and specificity (100%) to FP-CIT in this group (sensitivity 90%, specificity 76%); the two modalities agreed on 68% of cases enrolled in the study. It should be noted, however, that the study included thirteen patients with FTD and PSP among their non-DLB group, and such conditions capable of producing false positive FP-CIT results (Vlaar *et al.*, 2008; Morgan *et al.*, 2012). The two Italian groups employed different methods of determining whether a HMR finding was abnormal. The

Tiraboschi *et al.* (2016) study used the 1.6 cut-off used by Yoshita *et al.* (2006), while Treglia *et al.* (2012) considered myocardial uptake abnormal if it was more than two standard deviations below the population mean; this corresponded to a threshold of 1.55.

3.4.5 HMR cut-off

A weakness of the evidence surrounding MIBG is that there does not appear to be a clear consensus regarding what represents a normal and an abnormal scan. This is in contrast to the established visual rating system used in FP-CIT interpretation (Benamer *et al.*, 2000).

As previously discussed, semi-quantification of cardiac MIBG uptake is achieved through calculation of the HMR; the MIBG uptake in a heart ROI is divided by uptake in the reference mediastinal ROI. An MIBG scan is deemed abnormal when HMR falls below a certain value. If a cut-off were too low, DLB cases with lesser degrees of denervation (and thus higher HMR values) might not fall under the threshold, producing a false negative result; too high a threshold, and non-DLB cases with uptake at the lower end of normal range could produce false positive results.

MIBG studies to date have varied in their approach to the threshold between abnormal and normal scans. Only a handful of studies report *a priori* HMR cut-offs, citing values of 1.56-1.60 derived from the mean values of older patients in local databases (Estorch *et al.*, 2008; Marquié Sayagués *et al.*, 2010; Tiraboschi *et al.*, 2016). Several Japanese studies calculated *post hoc* cut-offs between 1.68 and 2.10 (Yoshita *et al.*, 2006; Wada-Isoe *et al.*, 2007), or sought only to identify a significant difference between DLB and non-DLB group mean values (Watanabe *et al.*, 2001; Oide *et al.*, 2003; Noguchi-Shinohara *et al.*, 2009). Others characterised abnormal scans as those with HMR values falling two or three standard deviations below the control population mean (Hanyu *et al.*, 2006; Novellino *et al.*, 2010). Although two studies did employ a visual rating system in parallel with HMR analysis, semiquantification was the preferred method of analysis in discussion (Yoshita *et al.*, 2015; Tiraboschi *et al.*, 2016).

The observed variation in HMR cut-off practices presents difficulty in translating MIBG into clinical practice, where nuclear medicine departments may not have access to a control population against which to compare HMR values in people with dementia. The range of reported *post hoc* values might also suggest that different control populations may possess factors influencing HMR,

and therefore MIBG utility. This is particularly significant when one considers that the vast majority of MIBG research to date has taken place in Japanese populations. Nakajima *et al*. (2012) attempted to account for such variation across centres using a calibration method for ten participating departments, a practice extended to European centres in a recent study (Verschure *et al.*, 2017), but MIBG otherwise currently lacks the standardised approach to reporting possessed by FP-CIT.

3.4.6 MIBG Conclusions

MIBG shows promise as an accurate biomarker in distinguishing DLB from AD. Studies in both Japan and Europe have suggested encouraging, but variable rates of sensitivity and specificity (87-100%); several of these are reported rates superior to those seen in FP-CIT (Estorch *et al.*, 2008; Noguchi-Shinohara *et al.*, 2009; Novellino *et al.*, 2010). However, the largest sample size in the field to date reported relatively inferior sensitivity (69%) and comparable specificity (89%) to FP-CIT (Yoshita *et al.*, 2006).

The evidence surrounding MIBG has important limitations that challenge integration into routine clinical practice, which future research should seek to address. Study samples to date have largely failed to accurately represent clinical populations, particularly with respect to inclusion of subjects with comorbidities and those with medications suspected to interfere with MIBG uptake. No studies to date have researched MIBG in a UK population. MIBG and FP-CIT have not been extensively compared in concurrent populations, despite the potential value of doing so to clinical decision making. This is further complicated by the absence of a consensus regarding a clear HMR threshold to distinguish an abnormal MIBG scan from a normal scan.

3.5 Summary

Several biomarkers exist to assist clinical decision making in DLB diagnosis, each with their respective strengths and weaknesses, but FP-CIT and MIBG in particular are capable of distinguishing DLB from AD and from other non-DLB pathologies. FP-CIT is supported by a developed evidence base, comprised by studies comparing its utility with both clinical and neuropathological DLB diagnosis. Although reliable and widely available, it does have weaknesses that include a suboptimal sensitivity. Other concerns surround its accuracy in patients without parkinsonian features and in subjects in the early stages of their illness.

Some MIBG studies have reported sensitivity and specificity superior that of FP-CIT, but despite its equivalent status as an indicative biomarker in the fourth consensus criteria (McKeith *et al.*, 2017), the evidence base surrounding MIBG is less well developed than that of FP-CIT. The role of interfering medications and comorbidities, as well as the practical considerations such as the HMR threshold in differentiating normal and abnormal scans, must be further explored before MIBG is established alongside FP-CIT as a routine biomarker for differentiating DLB from AD.

Chapter 4

A study of the frequency of DLB cases in NHS clinical services - aims, hypotheses and methods

4.1 Aims

The aim of this study was to determine the prevalence of DLB in a number of different NHS clinical services. Through subsequent detailed extraction of data from clinical case notes, it also aimed to compare the clinical pathway to diagnosis in DLB cases, to those with non-DLB dementia diagnoses; in particular, factors including the time to make a diagnosis, and the degree of clinical contact, both before and after diagnosis. As a measure of frequency, we examined the number of DLB cases seen as a proportion of all dementia cases seen during an 18-month period (prevalence). We also measured the proportion of new DLB diagnoses, made during the same 18-month period, as a proportion of all new dementia diagnoses made during this time (incidence).

Our intention was to better understand DLB prevalence using a large, representative clinical population using a systematic methodology. Time to diagnosis and degree of clinical contact among a large DLB cohort were explored in order to understand the implications of DLB diagnosis to routine clinical care. Rates of diagnostic revision, and clinical characteristics of patients undergoing diagnostic revision, were investigated with the intent of exploring opportunities to improve DLB recognition and diagnosis in routine NHS care.

4.2 Objectives and hypotheses

4.2.1 Objective 1

Our first objective was to determine the prevalence and incidence of DLB in NHS services through clinical note screening. Based on previous literature, discussed in Chapter 2, suggesting that many DLB cases were not recognised during life, we hypothesised that:

- DLB would represent less than 5% of all dementia cases seen in Psychiatry of Old Age (POA) services;
- that DLB prevalence would vary across services examined;
- DLB would be equally prevalent in men and women;
- DLB would be equally prevalent in patients of different ages.

4.2.2 Objective 2

Our second objective was to determine clinical factors related to DLB diagnosis and matched non-DLB dementia controls through detailed case note analysis. Because of previously suggested difficulties in making an accurate DLB diagnosis we hypothesised that:

- The diagnostic process would be significantly longer for DLB diagnoses than for non-DLB diagnoses.
- A higher proportion of patients with DLB than non-DLB patients would receive one or more alternative dementia diagnoses prior to their final diagnosis.
- Patients with DLB would undergo more clinical contacts before and after diagnosis than their non-DLB counterparts.

Although an early objective of the study was to determine the mortality rate in our screening population, it became immediately apparent early in the process that the recording practices for death were extremely inconsistent and incomplete in all three services. Data collected on mortality was not therefore included in analysis.

4.3 Methods

This study was conducted in two phases. Phase 1 was a retrospective cohort study, in which the clinical records of all patients seen in three POA services were examined and limited demographic information retrieved for each patient.

Phase 2 was a matched retrospective cohort study. DLB patients identified during Phase 1 were approached for consent to a detailed case note review and analysis of all clinical interactions during their contact with services. Non-DLB controls, matched for gender, age (± 3 years) and MMSE (± 5 points) at the point of diagnosis, were also approached for consent. Ethical approval for both phases was granted by Newcastle and North Tyneside NHS Research Ethics Committee (REC). Permission was granted by the Confidentiality Advisory Group (CAG) to access clinical data without individual patients' consent for Phase 1 of the study. This was important in ascertaining prevalence accurately, as approaching every individual seen in services would have been impractical and subjected our findings to selection bias and survivor bias.

4.3.1 Phase 1 - Case screening

4.3.1.1 Study design

Phase 1 was a retrospective cohort study of POA services in North East England. The study team retrospectively reviewed notes from all patients seen in three services in North East England over an 18-month period between 1st January 2013 and June 30th, 2014. This period of time was chosen as it would allow sufficient time for additional investigations (e.g. FP-CIT) or revision of diagnosis where necessary. A longer timeframe would have been both unfeasible and may have also been susceptible to changes in the structure and personnel in participating services.

Services were selected by the research team on the basis of a number of factors;

- **Clinicians' consent** to participate in the exercise. Although CAG afforded access to patient notes, the research team felt it good practice to approach the clinicians leading the services and gain their permission to access and analyse their caseloads. None of the services approached declined permission to take part in the study.
- The **volume of patients** attending each participating service. The research team hoped to identify at least 30 patients to participate in Phase 2 of the study, estimating that approximately 50% of patients would consent to detailed case note review. We hypothesised that DLB would comprise less than 5% of all dementia diagnoses, so estimated that 60 DLB cases would be identified amongst a minimum of 1200 dementia cases. A DLB sample size of 60 in an overall dementia population of 1200 would, using Wilson's method (Brown, DasGupta and Cai, 2001), provide a 95% confidence interval of 3.8 to 6.4% for the actual DLB prevalence.
- The **generalisability of the services** to those existing in other NHS trusts and regions. The research team felt it important that the wide range of service structures, clinical skills and patient populations in the NHS nationally were represented in the study cohort.

The case notes of patients from three services, operating within two healthcare trusts each and comprising several components, were therefore screened;

- Newcastle upon Tyne memory services (Northumberland Tyne and Wear Foundation Trust); this comprises a large group of integrated memory clinics and associated Community Mental Health Teams (CMHTs). It also includes a tertiary Lewy body disease

clinic and a mental health day hospital. It services a predominantly urban population and is closely associated with Newcastle University.

- St George's Hospital, Morpeth (Northumberland Tyne and Wear Foundation Trust); this service comprises a group of memory clinics and paired CMHTs affiliated with a rural psychiatric hospital. It serves a predominantly rural population.
- North Tyneside Memory Clinic and associated CMHTs (Northumbria Healthcare NHS Foundation Trust); this service comprises a group of memory clinics and CMHTs affiliated with a District General Hospital. This service also includes a specialist team addressing behavioural and psychological symptoms of dementia in local nursing homes. It serves a mixed rural and urban population.

For the purposes of analysis and discussion, these three services were anonymised; we have referred to "*Services A, B and C*" throughout the remainder of this thesis.

Informatics services at participating trusts provided the research team with lists of patients seen during the 18-month screening window. These included all forms of contact, including scheduled clinical appointments, hospital liaison reviews, day hospital and inpatient reviews, home visits, information groups and emergency assessments. Contact was led by a range of multidisciplinary professionals that included psychiatrists, nurses, social workers, psychologists, support workers, physiotherapists, and occupational therapists, amongst others. Although patients may not have been reviewed by a psychiatrist during the 18-month screening window, all patients under review by participating services had a named psychiatric consultant coordinating their care. Consultant caseloads therefore provided the most accurate and extensive record of patients seen by multidisciplinary services. Although only patients with dementia seen within the screening window were included, the entirety of each patient's clinical notes were reviewed, as in some cases it was expected that diagnostic revision would occur after the end of the screening period. Follow-up occurred for a minimum of one year.

The research team conducting the collection of data for both Phases 1 and 2 comprised;

- Joseph Kane (JK), Clinical Research Associate and PhD candidate, Newcastle University.
- Sally Barker (SB), Clinical Research Nurse, Newcastle University.
- Neil Fullerton (NF), Clinical Trials Officer, National Institute for Health Research Clinical Research Network: Dementias and neurodegeneration (DeNDRoN)

Both JK and SB had prior clinical experience in working with patients with dementia, in their respective roles as a training psychiatrist and district nurse. NF, through his role as Clinical Trials Officer, had experience in research studies recruiting patients with dementia. This role also included note screening and data collection. None of the research team conducting data collection for Phases 1 and 2 had been previously been employed by any of the participating services.

The research team accessed the clinical notes of each patient identified by each informatics list. Patients with a clearly documented diagnosis of dementia were recorded. Patients who had previously documented that they did not wish their notes to be accessed by research teams and those without a history of dementia recorded during the screening window were excluded from this process, such as patients with mild cognitive impairment or delirium and those with psychotic, affective and addiction disorders. Cases initially given these non-dementia diagnoses, but later diagnosed with dementia within the screening dates were included.

From the records of patients with a diagnosis of dementia, gender, date of birth and subtype diagnosis were recorded. During the screening each patient was categorised as either prevalent (initial dementia diagnosis prior to 1st January 2013 but still being seen in the service) or incident (dementia newly diagnosed between 1st January 2013 and 30th June 2014). A patient's prevalent/incident status was not affected by subtype diagnosis revision; if the diagnosis of a prevalent case with AD was revised to DLB during the 18-month screening period, the case would be categorised as a prevalent DLB case. Mini-mental state score (MMSE) at the point of dementia diagnosis was recorded for matching purposes.

Figure 4.1 Examples (simulated data) of patient information recorded during Phase 1

Surname	Forename	Patient Number	DOB	Date seen	Gender	Consultant	Diagnosis	Prevalent/ Incident	MMSE at diagnosis
SMITH	John	1111111	01/01/1920	01/01/2013	M	Dr Jones	AD	Prevalent	23/30
WHITE	Jane	1111112	01/02/1920	01/01/2013	F	Dr Jones	DLB	Incident	21/30

Some patients attended more than one participating service during the 18-month screening window. Each patients' name was therefore recorded in order to identify duplicate records, so that the same subject was not inadvertently included as two separate subjects. In such cases, patients were included amongst the cohort corresponding to the service in which they were first seen during the screening window. The duplicate records were then removed from analysis.

Patients selected for approach for Phase 2 also had their address and trust identification number temporarily recorded for administrative purposes and communication with clinical teams. Dementia subtype diagnosis was determined by the primary working diagnosis. Mixed DLB/AD cases were documented as DLB. Where primary dementia subtype diagnosis was unclear from the documentation available, the diagnosis was recorded as "*Dementia not otherwise specified*".

Patients with a recorded diagnosis of one subtype during the screening window, but in whom the diagnosis was later revised after the screening window (June 2014) had their most recent primary diagnosis recorded. Date of death, where available, was recorded in patients who had deceased.

Prior to analysis, dementia subtypes were recoded to two groups; DLB and non-DLB.

4.3.1.2 Statistical analysis

Data were entered into a Microsoft Excel spreadsheet. This data, once anonymised and cleaned, was imported to Statistical Package for Social Sciences (SPSS, version 24) for statistical analysis.

Prevalence was calculated as the percentage of DLB cases amongst of all dementia cases seen by services within the 18-month window. Incidence was calculated as the number of incident DLB cases as a proportion of all incident dementia cases. Confidence intervals for both prevalence and incidence were calculated using the Wilson method (Brown, DasGupta and Cai, 2001).

For a large number of cases, the exact date of dementia diagnosis was not available, and it was not possible therefore to determine patient age at the time of dementia diagnosis. In lieu of patient age at dementia diagnosis, the age of each patient on 1st October 2013 (the midpoint of our screening window) was calculated. The mean ages of the DLB and non-DLB cases were compared using Student's independent t-test.

Thereafter, patients were stratified into age groups of five years and the Mantel-Haenszel χ^2 test used to test for a relationship between age and prevalence. The χ^2 test was used to compare gender prevalence between DLB and non-DLB groups. For each test statistic, a probability value of $p<0.05$ was regarded as significant.

4.3.2 Phase 2 - Detailed case note review

4.3.2.1 Study design

Phase 2 was an observational retrospective matched cohort study. It used data retrieved from the clinical notes of patients identified during Phase 1. All patients with a diagnosis of DLB, first recorded during the 18-month screening period were considered for Phase 2. Patients in whom a non-DLB dementia subtype was recorded during the screening period, but whose diagnosis was revised to DLB after the screening period, were also included in this group. A member of the multidisciplinary team currently treating the patient was consulted regarding suitability to approach for consent. In cases where the patient had been discharged from secondary services, the clinician whom the patient had most recently seen was consulted regarding suitability.

Eligible DLB cases were then approached by the patients' clinical team, contacting patients via a letter that included a form to "*opt out*" of the study and any further contact with the research team. Patients who neither returned the "*opt out*" form, nor contacted the research team to express further interest, were contacted by telephone (SB and JK). Patients expressing interest in taking part were visited by the research team (SB and JK) and, where applicable, obtained informed consent. In cases where patients did not have capacity to consent to participation, but the patient demonstrated a willingness to participate, a consultee provided consent on their behalf.

We adopted an individual matching methodology; recruited DLB cases were matched to the next non-DLB patient seen consecutively within the respective service, matched for gender, age (± 3 years) and MMSE (± 5 points). Where the first matched non-DLB patient declined participation, the next consecutive patient satisfying matching criteria was approached, and so on.

The research team obtained the clinical records for each recruited participant. Only the medical notes from participating organisations were accessed; where a patient underwent clinical contact in another organisation (such as an Accident & Emergency department (A&E) attendance in an acute trust), this was only recorded if it was documentation of the event was made in the participating services' notes. We collected data from the first point of contact in the clinical notes (often GP consultation and referral to secondary services) to the day of Clinical Record Form

(CRF) completion. The duration of time over which this data was collected was calculated in both DLB and non-DLB groups.

The team (JK, SB) extracted detailed data to a CRF. This included several sections;

1. **Personal details**; age, gender, ethnicity; status as prevalent/ incident case.
2. **Diagnostic details**; date of referral, date first seen, date of first diagnosis; date of subsequent diagnoses; the specialty of the first clinician to make each of these diagnoses was also noted.
3. **Clinical symptoms;** the presence of each central, core, suggestive and supportive DLB symptom (as defined by the third consensus criteria (McKeith *et al.*, 2005)) were recorded.

   The date of onset was each symptom was estimated and recorded (e.g. where a *"two-month history of depression"* was noted in clinical documentation, the date two months prior to the clinic date was entered). Where no duration was noted for such symptoms, the date of the clinical documentation referring to the symptom was noted. Further qualitative information on each symptom was noted for the purposes of the validation process.

   The third consensus criteria refers to two symptom groups rather than individual symptoms; "*spontaneous features of parkinsonism*" and "*severe autonomic dysfunction*". For each of these groups, the CRF recorded the presence and date of onset of individual symptoms in addition to the presence and onset of symptom groups. Bradykinesia, rest tremor, rigidity, shuffling gait and postural instability comprised features of parkinsonism; constipation, orthostatic hypotension, urinary incontinence and comprised severe autonomic dysfunction. The presence of one or more symptom was noted to have indicated the presence of the symptom group, and the earliest date of onset was noted to have indicated the date of onset of the symptom group.

4. **Past medical history**; significant health conditions, hospitalisations (for both psychiatric and physical health presentations), A&E presentations and operations were documented with date of onset and qualitative information.
5. **Drug history;** drug names, doses and frequency at the point of dementia diagnosis were documented. Start dates, end dates, side effects and efficacy for each drug were also noted.

6. **Drug changes;** changes made since contact with secondary services, including changes in preparation or dose, were recorded, together with side effects, start dates, end dates and efficacy. Where titration of a drug occurred, the highest daily dose of each agent was noted.
7. **Family history** of significant chronic health conditions were recorded.
8. **Basic physical information**, including height, weight, pulse, standing and lying blood pressure were recorded. Physical examinations, and the findings of such examinations were also documented.
9. **Investigations and tests** were recorded and dated, including neuropsychology tests, blood tests, neuroimaging and other imaging investigations, electrocardiograph, EEG, lumbar puncture and any other documented investigation.
10. **Clinical contact** prior to dementia diagnosis was recorded, along with information on the date, clinic type (including home visits), clinicians involved and whom, if anyone, accompanied the patient during the appointment. Referrals made to other services, including voluntary sector services, during these appointments were noted, as were documented instances of carer stress.
11. **Post-diagnostic management** strategies were noted in detail. Pharmacological management, referral to other services, voluntary sector referral, recommendation for driving assessment, medicolegal advice amongst other treatment strategies were documented.
12. **Post-diagnostic clinical contact,** with information on date, clinic type, clinicians involved and persons accompanying the patient to each appointment documented.

Each CRF also included a brief (approximately 200 word) clinical vignette outlining the clinical course and symptom profile. These were written by JK and SB and were prepared primarily for the purposes of assisting with the case validation by independent experts. JK completed 64 CRFs, SB completed 35 CRFs. JK and SB completed 5 CRFs together to ensure that data was collected and recorded in a consistent manner.

4.3.2.2 Case validation

Each DLB case and non-DLB control was validated by two independent clinical experts, neither of whom had had prior clinical contact with the patient (JK, Professor John O'Brien (JOB), Dr John-Paul Taylor (JPT), Professor Ian McKeith (IMcK)). The experts, using all the information contained in each CRF, systematically applied diagnostic criteria for major subtypes for each case. They then documented the rationale for a final diagnosis. Where the final diagnosis returned by raters 1 and 2 differed (in 25 cases), a third expert (IMcK, JPT) was asked to review and validate diagnosis. Where, even after a third expert was consulted, no two clinical experts agreed on diagnosis (which occurred in 3 cases), raters 1, 2 and 3 reviewed the case in question and together came to a consensus on diagnosis. In cases where the consensus diagnosis returned was not one of dementia (e.g. MCI), the case in question was excluded from analysis (n=1). In cases in which a non-DLB case fulfilled criteria for DLB, or vice versa, the case in question was excluded from analysis (n=1).

This method has been shown to be an acceptable, practical and suitably accurate alternate to the *post-mortem* gold standard for DLB diagnosis (McKeith *et al.*, 2000b).

4.3.2.3 Data entry

CRF data was transposed to a bespoke Microsoft Access database by members of the research team (JK, SB). Ten CRFs were selected at random and compared with transposed entries. An error rate of below 5% was deemed satisfactory at the start of the process. A total of 28 errors, each in different data fields, were identified and rectified during this process, representing less than 0.01% of all data entered.

4.3.2.4 Data analysis

Access database data were imported into SPSS for data analysis. Continuous variables were compared using independent t-test and categorical variables compared with χ^2 test. Wilcoxon signed-rank test was used to compare non-parametric matched samples and Mann-Whitney U test used for non-parametric independent samples. For each test statistic, a probability value of $p<0.05$ was regarded as significant. Bonferroni correction for multiple comparisons was not used; Armstrong (2014) advises against the use of such tests in *post hoc* testing, particularly in exploratory studies such as this one. Results are described in the next chapter.

Chapter 5

A study of the frequency of DLB cases in NHS clinical services - results

5.1 Recruitment and matching

5.1.1 Phase 1

A total of 5 569 cases, each of whom had attended the three participating services between 1st January 2013 and June 2014, were screened during Phase 1. Of these cases, 2 575 (46.2%) had a recorded diagnosis of dementia, of which 144 cases had a DLB diagnosis and 2 431 a non-DLB diagnosis.

5.1.2 Phase 2

Every individual identified during Phase 1 as having a diagnosis of DLB was approached for participation in Phase 2. Of this group, 52 patients gave consent for the research team to review and collect data from their clinical records. Fifty-four matched participants were also recruited for Phase 2.

Figure 5.1 provides a complete account of case identification, recruitment and matching for both Phase 1 and Phase 2.

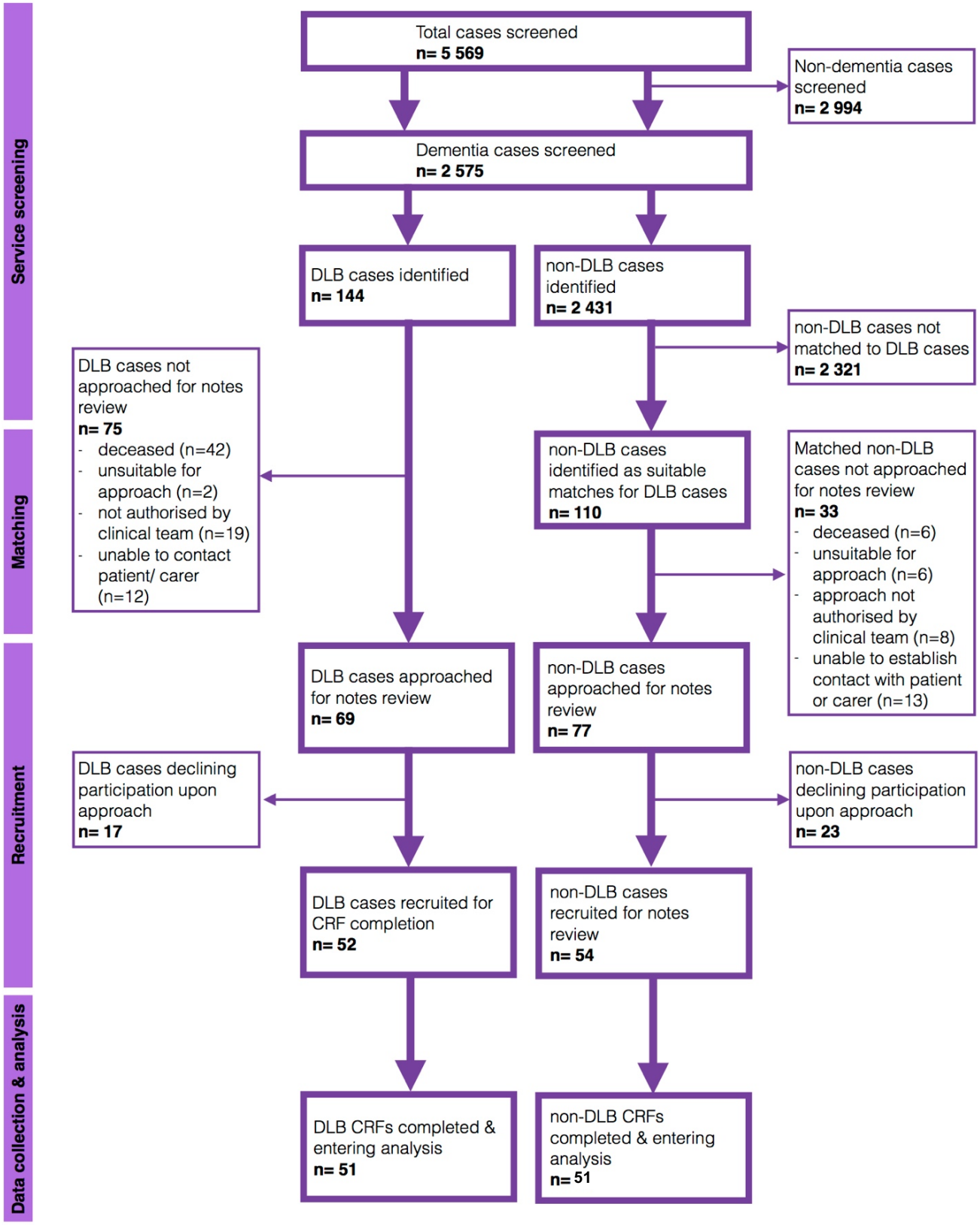
5.2 Phase 1 results

The results presented in *Table 5.1* are divided into prevalent and incident cases, as previously described, whereby prevalent cases are all dementia cases seen, and incident cases are those with a new diagnosis made during the 18-month screening window.

Table 5.1 DLB prevalence and incidence

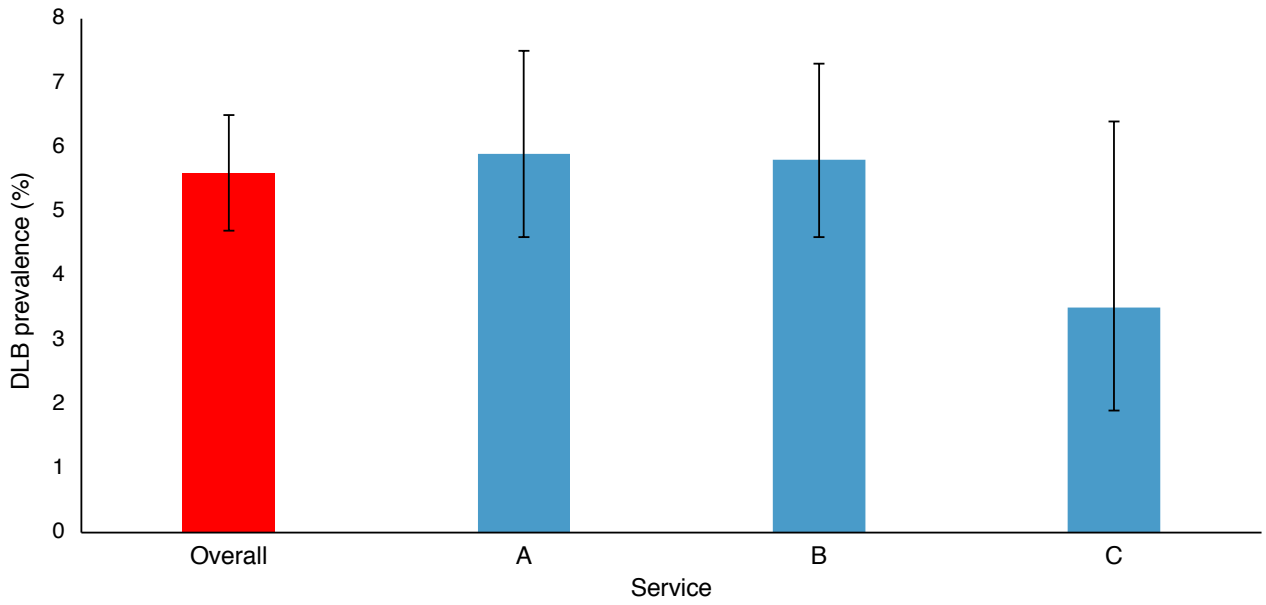
Service	**Dementia (all subtypes)**		**DLB**			
	Prevalent	Incident	Prevalent	% of prevalent dementia cases (95% CI)	Incident	% of incident dementia cases (95% CI)
A	1 115	548	66	5.9% (4.6-7.5%)	35	6.4 (4.6-8.8%)
B	1 178	637	68	5.8% (4.6-7.3%)	36	5.7 (4.1-7.7%)
C	282	106	10	3.5% (1.9-6.4%)	4	3.8 (1.5-9.3%)
Total	2 575	1 291	144	5.6% (4.7-6.5%)	75	5.8 (4.6-7.2%)

Figure 5.1 Flow diagram of patient identification, recruitment and matching for Phases 1 and 2



The clinical prevalence of DLB (n= 144) in our screened (n= 2 575) population was 5.6% (95% CI 4.7-6.5%) *(Table 5.1)*. Prevalence ranged from 3.5% (95% CI 1.9-6.4%) to 5.9% (95% CI 4.6-7.5%). When incident cases alone were analysed, DLB cases (n=75) comprised 5.8% (95% CI 4.6 - 7.2%) of dementia cases (n=1 291) *(Figure 5.2)*. No significant difference in prevalence (χ^2 =2.54, p = 0.28) nor in incidence (χ^2 =1.17, p = 0.56) was observed between the three services.

Figure 5.2 DLB prevalence across participating services



In prevalent cases, DLB was significantly more common in men than in women (χ^2 = 24.0, p<0.01). Males comprised 55.6% (95% CI 47.1 - 63.8%) of DLB cases, but only 36.5% (95% CI 34.6 - 38.4%) of non-DLB cases. There was also a significant difference when incident cases alone were compared (χ^2 = 6.2, p=0.01) (*Table 5.2*).

There was no significant difference in age between DLB and non-DLB dementia when considering either prevalent (80.7 ± 7.4 *vs* 81.9 ± 7.6; (t (2573)= -1.849, p =0.65) or incident (81.2 (± 7.1) v 81.7 ± 7.8; t. (1289)= -0.55, p =0.58) cases *(Table 5.2)*.

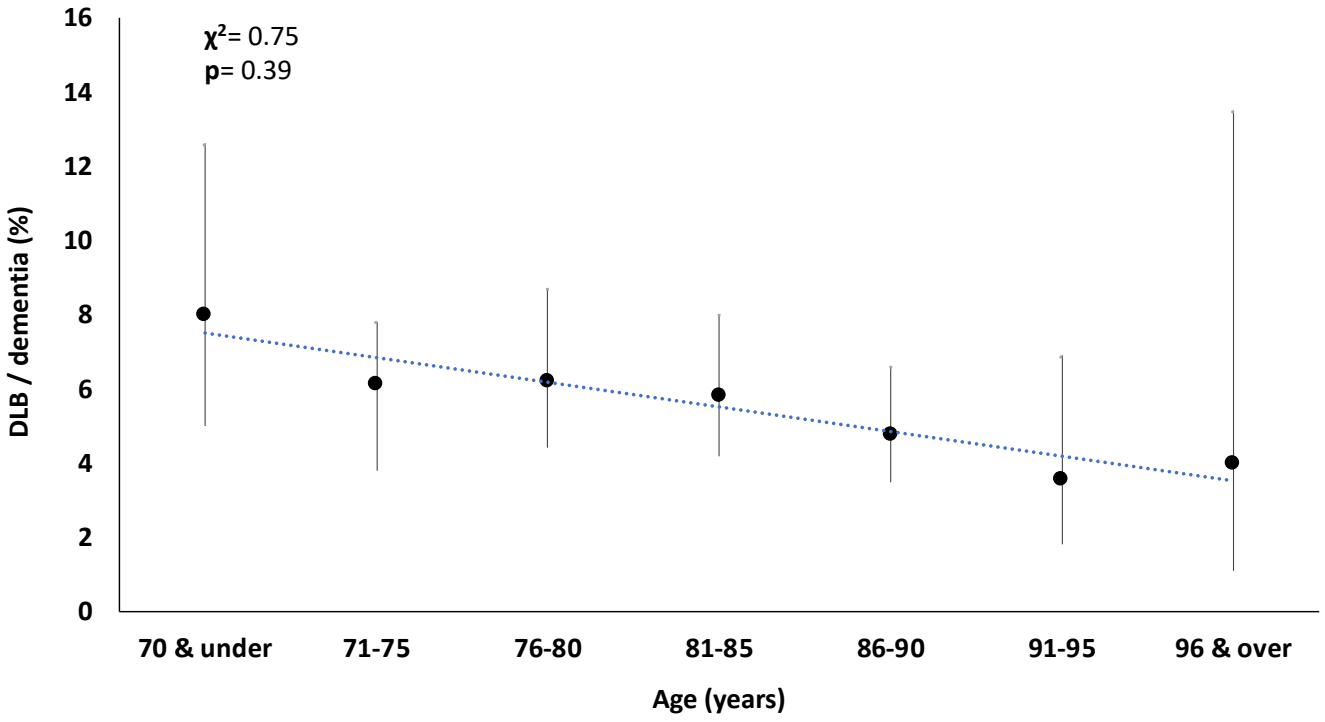
The proportion of DLB cases represented among dementia cases in various age groups was also assessed *(Figure 5.3).* DLB cases were observed at higher rates in younger age groups presenting to clinical services (χ^2= 5.03, p=0.03); prevalence comprised 7.9% (95% CI 4.9-12.4%) of cases in patients 70 and under, but only 3.0% (95% CI 1.4-6.3%) of cases in patients between the ages of 91

and 95. No significant correlation was identified between age and DLB incidence (χ^2=0.75, p =0.39).

Table 5.2 Age and gender of DLB and non-DLB patients

	DLB	non-DLB	p
Age at screening (years)(SD)			
Prevalent	80.7 (± 7.4)	81.9 (± 7.6)	0.65
Incident	81.2 (± 7.1)	81.7 (± 7.8)	0.58
Gender M/F (% M)			
Prevalent	80/64 (55.6%)	859/1572 (36.5%)	**<0.01**
Incident	38/37 (50.7%)	442/774 (36.3%)	**0.01**

Figure 5.3 DLB prevalence and age



5.3 Phase 2 results

5.3.1 Case matching

Every patient identified as having a DLB diagnosis during Phase 1 was considered for approach for Phase 2. Seventy-five DLB cases were not approached; 42 having deceased between Phases 1 and 2, and 21 cases considered by either the treating clinician or research team as unsuitable for approach (*Figure 5.1*). In 12 cases, the research team was unable to contact the patient or a suitable consultee. Once an individual declined participation in the second phase of the study we disposed of their matching data. This, together with our failure to adequately cross-reference databases from Phase 1 and Phase 2, meant that we were unable to compare patients enrolled in Phase 2 and those that were not.

DLB and non-DLB groups were well matched on an individual basis by gender, age at dementia diagnosis, MMSE at dementia diagnosis and duration of follow up *(Table 5.3).*

Table 5.3 Demographic details of cases

	Gender M/F (%M)	Age at recruitment (years)(SD)	MMSE score at recruitment (SD)	Duration of follow up (days)(SD)
DLB (n=51)	31/20 (60.8%)	77.6 (± 7.3)	21.6 (± 5.1)	1365 (± 811)
non-DLB(n=51)	31/22 (58.5%)	77.7 (± 7.3)	20.7 (± 4.7)	1215 (± 928)

5.3.2 Revision of diagnosis

Throughout Phase 2 of data analysis, a number of time points at which to compare different groups were employed;

- "*Initial dementia diagnosis*" refers to the date of dementia diagnosis for DLB and non-DLB patients;
- "*Final dementia diagnosis*" the date after which there was no recorded revision of dementia subtype, and;
- *"Final data collection"* the date after which the research team no longer recorded clinical data from the patient's notes for use in the study.

Table 5.4 Revision of initial diagnosis

	DLB (n=51)	non-DLB (n=51)	p
Patients whose dementia diagnosis was revised/ changed n (% of cases)	20 (39%)	1 (2%)	**<0.01**

Twenty patients with DLB (39%) were initially diagnosed with an alternative dementia subtype (AD n=14, VaD n=2, mixed AD/VaD n=3, PDD n=1). This proportion was significantly higher than that observed amongst patients with non-DLB subtypes (2%; $\chi^2 = 21.6$, $p<0.01$).

5.3.3 Time to diagnosis

The case notes of participants were reviewed in detail, and relevant time points, including date of referral, date of first assessment, date of initial diagnosis, and date of final diagnosis, extracted. In some instances, one of more of these data were not detailed in the clinical documentation. Thus, such cases could not contribute to the comparison of respective timescales, such as those represented by *Table 5.5.* The sample sizes (n), representing the number of patients for whom valid data was available for each measurement, are therefore provided in each table.

All data comprising time to diagnosis calculations had skewed distributions; Wilcoxon signed-ranks test and Mann Whitney U test were therefore used to compare data between groups.

Table 5.5 Time from referral to diagnosis

	DLB			non-DLB			p
	n	Mean (days)(SD)	Median (days)	n	Mean (days)(SD)	Median (days)	
Referral to first assessment	44	71 (± 90)	35	49	62 (± 53)	47	0.64
Referral to initial dementia diagnosis	42	324 (± 504)	170	47	47 (± 503)	146	0.94
Referral to final diagnosis	45	567 (± 656)	265	48	48 (± 542)	154	**0.04**

Table 5.6 Time from first assessment to diagnosis

	DLB			non-DLB			p
	n	Mean (days)(SD)	Median (days)	n	Mean (days)(SD)	Median (days)	
First assessment to initial dementia diagnosis	45	251 (± 487)	98	48	256 (± 499)	90	0.98
First assessment to final diagnosis	48	481 (± 623)	207	49	291 (± 532)	90	**0.02**

Table 5.7 Time from initial dementia diagnosis to final dementia diagnosis

	DLB			non-DLB			p
	n	Mean (days)(SD)	Median (days)	n	Mean (days)(SD)	Median (days)	
Initial dementia diagnosis to final dementia diagnosis	51	287 (± 464)	0	50	46 (± 193)	0	**<0.01**

The time from referral to final diagnosis (z= -2.01, p=0.04) *(Table 5.5)*, first assessment to final diagnosis (z=-2.38, p=0.02) *(Table 5.6)* and initial dementia diagnosis to final dementia diagnosis (z=-3.32, p<0.01) *(Table 5.7)* were all significantly higher in the DLB group compared to the non-DLB group. These relationships are unsurprising as 98% of non-DLB patients underwent no revision of subtype diagnosis and thus the value for this parameter was zero *(Table 5.4).*

Our significant findings regarding longer time to diagnosis in DLB patients led us to consider factors that might contribute to these findings, one of which might be the need for additional investigations, especially scans, that might cause delays for logistical reasons. We therefore compared the same data points for DLB patients who had FP-CIT imaging as part of their diagnostic work-up with DLB patients that did not.

Table 5.8 FP-CIT and time from referral to diagnosis

	DLB patients who had FP-CIT imaging				DLB patients who did not have FP-CIT imaging				p
	n	Mean (days)(SD)	Median (days)	Mean rank	n	Mean (days)(SD)	Median (days)	Mean rank	
Referral to first assessment	27	90 (± 101)	47	26	17	40 (± 58)	22	17	**0.02**
Referral to initial dementia diagnosis	25	451 (± 617)	211	26	17	137 (± 133)	100	15	**<0.01**
Referral to final diagnosis	27	611 (± 735)	245	23	18	501 (± 528)	358	23	0.90

57% (n= 29/51) of recruited patients with DLB had FP-CIT scans during their contact with secondary care. Again, data regarding date of referral, assessment and diagnosis were not available for every patient, so sample sizes varied between different measurements of time (e.g. referral to first assessment).

There was a significantly longer period of time between referral and initial dementia diagnosis between DLB subjects who had FP-CIT scans (median 211 days) and those who did not (median 100 days; U= 110, z=-2.63 p<0.01). We also observed a significantly longer period of time between referral and first assessment in those who attended a FP-CIT scan (median 47 days) compared to those who didn't (22 days; U = 130, z=-2.40, p=0.02) *(Table 5.8)*.

Despite this, we observed a non-significant trend for those who had FP-CIT to experience a shorter time from referral to final DLB diagnosis.

Table 5.9 FP-CIT and time from initial diagnosis to final diagnosis

	DLB patients who had FP-CIT imaging				DLB patients who did not have FP-CIT imaging				p
	n	Mean (days)(SD)	Median (days)	Mean rank	n	Mean (days)(SD)	Median (days)	Mean rank	
Initial dementia diagnosis to final dementia diagnosis	29	273 (± 436)	0	27	22	305 (± 509)	36	25	0.63

No significant relationship was observed between FP-CIT status and time from initial dementia diagnosis to final dementia diagnosis (*Table 5.9*).

Table 5.10 Revision of initial diagnosis in patients who had FP-CIT imaging

	DLB patients who had FP-CIT imaging (n=29)	**DLB patients who did not have FP-CIT imaging** (n=22)	**p**
Patients whose dementia diagnosis was revised/ changed n (% of cases)	11 (38%)	9 (38%)	0.98

Of DLB patients who had FP-CIT imaging, eleven (38%) had their dementia subtype diagnosis revised during their contact with secondary services. This was not significantly different to the proportion of DLB patients who did not have FP-CIT imaging and whose initial subtype diagnosis was later revised ($\chi^2 = 0.001$, p=0.98) (*Table 5.10*).

In summary, patients with DLB who had FP-CIT scans experienced a significantly longer period of time from referral to initial assessment, and from referral to initial dementia diagnosis, than those not referred for FP-CIT. However, FP-CIT was not associated with a longer period from referral to final diagnosis. Overall, no significant difference was observed in the rate of diagnostic revision in DLB patients who had FP-CIT scans than those who didn't have such scans.

5.3.4 Clinical contact

We will now compare aspects of the clinical care received by DLB and non-DLB groups, examining clinical contact firstly in the time prior to their dementia diagnosis, and secondly throughout the entirety of their time spent in secondary care.

Overall, we found no significant relationship between the number of contacts with services prior to dementia diagnosis and DLB status *(Table 5.11).* However, when we investigated the total number of contacts between participants and clinical services throughout their time in secondary care, we found that the total number of appointments was significantly higher in DLB patients than non-DLB patients (z=-2.82, p=0.01) *(Table 5.12)*.

Table 5.11 Total clinical appointments prior to initial dementia diagnosis

	DLB			**non-DLB**			**p**
	n	Mean (SD)	Median	n	Mean (SD)	Median	
Appointments (All healthcare professionals)	51	6.9 (± 12.5)	2	51	3.3 (± 4.2)	2	0.14

Table 5.12 Total number of clinical appointments throughout contact with services

	DLB			**non-DLB**			**p**
	n	Mean (SD)	Median	n	Mean (SD)	Median	
Appointments (All healthcare professionals)	51	24.1 (± 22.8)	17	51	14.5 (± 17.6)	10	**0.01**

These findings led us to consider the type of contact that each subject had had during their time with secondary care services. Patients were seen by a range of practitioners. These included medical professionals (such as psychiatrists, neurologists and geriatricians), as well as other members of the multidisciplinary team (specialist nurses, occupational therapists, physiotherapists, social workers, support workers and psychologists).

We first investigated the number of contacts between participants and each subspecialty prior to diagnosis. DLB patients had a significantly higher number of appointments with specialist nurses than non-DLB patients (U=133.5, z= -2.21, p=0.03) *(Table 5.13)*. No other significant differences were detected between DLB and non-DLB groups for the number of contacts with any other healthcare professional.

Table 5.13 Appointments with clinicians (prior to initial dementia diagnosis)

	DLB				**non-DLB**				**p**
	n	Mean (SD)	Median	Mean rank	n	Mean (SD)	Median	Mean rank	
Psychiatrist/ neurologist/ Geriatrician	51	2.6 (± 3.7)	2	57	51	1.5 (± 1.3)	1	48	0.14
Specialist nurse	15	6.1 (± 10.0)	3	28	28	2.3 (± 3.1)	1	19	**0.03**
Occupational therapist	12	3.0 (± 4.9)	1	7	2	3.5 (± 3.5)	4	9	0.52
Physiotherapist	3	2.3 (± 2.3)	1	2	0	n/a	n/a	0	n/a
Social worker	8	1.3 (± 0.5)	1	5	1	1.0	n/a	4	0.59
Psychologist	7	7.9 (± 6.8)	8	7	4	3.0 (± 2.3)	3	5	0.32
Support worker	0	n/a	n/a	n/a	2	2.0 (± 1.4)	n/a	2	n/a
Other clinician	0	n/a	n/a	n/a	0	n/a	n/a	n/a	n/a

When frequency of contact with individual specialties throughout the entirety of patients' time in secondary care was considered, DLB cases attended a higher number of appointments with medical practitioners (U=701, z=-3.68, p<0.01) and a higher number of appointments with specialist nurses (U= 424, z=-2.93, p<0.01) than non-DLB cases *(Table 5.14)*. No other significant relationships were identified between subtype diagnosis and the number of contacts with each professional group.

Table 5.14 Appointments with clinicians (overall)

	DLB				**Non-DLB**				**p**
	n	Mean (SD)	Median	Mean rank	n	Mean (SD)	Median	Mean rank	
Psychiatrist/ neurologist/ geriatrician	51	9.8 (± 6.7)	7	60	48	5.4 (±3.7)	5	39	**<0.01**
Specialist nurse	34	12.0 (± 12.0)	11	46	41	7.2 (±14.0)	3	31	**<0.01**
Occupational therapist	20	4.6 (± 8.8)	3	17	14	4.3 (±6.0)	2	18	0.81
Physiotherapist	12	3.2 (± 2.7)	3	9	6	3.3 (±2.9)	3	10	0.96
Social worker	14	2.6 (± 3.3)	2	9	3	1.7 (±0.6)	2	9	0.95
Psychologist	11	11.9 (± 8.2)	11	12	8	5.0 (± 5.5)	3	7	0.08
Support worker	5	3.4 (± 3.6)	1	6	9	6.0 (± 5.2)	6	8	0.33
Other clinician	3	1.3 (± 0.6)	1	2	1	4.0	n/a	4	0.16

After investigating the frequency of contacts between participants and clinician groups, we explored the proportion of patients in DLB and non-DLB groups receiving contact with each profession.

In the period prior to dementia diagnosis, we identified no relationship between DLB status and proportion of patients seen by psychiatrist, neurologist or geriatrician *(Table 5.15).*

Table 5.15 Proportion of DLB and non-DLB patients attending appointments with medical professionals prior to initial dementia diagnosis

	DLB (n=51) n (% of DLB cases)	**non-DLB (n=51)** n (% of non-DLB cases)	**χ^2 p**
Psychiatrist	44 (86%)	41 (78%)	0.30
Neurologist	4 (8%)	3 (6%)	0.68
Geriatrician	2 (4%)	2 (4%)	1.00
Allied health professional only	1 (2%)	6 (12%)	0.05

Of participants receiving contact from allied health professionals prior to diagnosis, a significantly higher proportion of DLB patients than non-DLB patients were seen by an occupational therapist (χ^2= 8.28, p<0.01) or social worker (χ^2 =5.97, p =0.02) *(Table 5.16)*. Contact with a specialist nurse was more common for non-DLB patients (55%) than DLB patients (29%) in the period prior to initial dementia diagnosis (χ^2 = 6.80, p = 0.01).

Table 5.16 Proportion of patients attending appointments with allied health professionals prior to initial dementia diagnosis

	DLB (n=51) n (% of DLB cases)	**non-DLB (n=51)** n (% of non-DLB cases)	**χ^2 p**
Specialist nurse	15 (29%)	28 (55%)	**0.01**
Occupational therapist	12 (24%)	2 (4%)	**<0.01**
Physiotherapist	3 (6%)	0 (0%)	0.08
Social worker	8 (16%)	1 (2%)	**0.02**
Psychologist	7 (14%)	4 (8%)	0.31
Support worker	0 (0.0%)	2 (4%)	0.16

When both contact with services before and after diagnosis were analysed, a significantly higher proportion of DLB patients (33%) than non-DLB patients (8%) were reviewed by a geriatrician at some point during their contact with services ($\chi^2 = 9.25$, $p<0.01$). Whilst a higher proportion of DLB patients than non-DLB patients had contact with a psychiatrist or neurologist during their time in services, the relationship between proportion of patients seen and subtype diagnosis was not statistically significant in either case *(Table 5.17)*.

Table 5.17 Proportion of patients receiving contact from medical clinicians (overall)

	DLB (n=51) n (% of DLB cases)	**non-DLB (n=51)** n (% of non-DLB cases)	**χ^2 p**
Psychiatrist	51 (100%)	48 (94%)	0.09
Neurologist	8 (16%)	3 (6%)	0.14
Geriatrician	17 (33%)	4 (8%)	**<0.01**

The significant trend for a higher proportion of DLB patients than non-DLB patients to see a social worker in the prediagnostic phase was also observed when all contacts were considered (28% vs 6%; $\chi^2 = 8.54$, $p<0.01$). No other significant relationships were identified between DLB status and contact with any other member of the multidisciplinary team *(Table 5.18)*.

Table 5.18 Proportion of patients receiving contact from allied health professionals (overall)

	DLB (n=51) n (% of DLB cases)	non-DLB (n=51) n (% of non-DLB cases	χ^2 p
Specialist nurse	34 (67%)	41 (80%)	0.12
Occupational therapist	20 (39%)	14 (28%)	0.21
Physiotherapist	12 (24%)	6 (12%)	0.12
Social worker	14 (28%)	3 (6%)	**<0.01**
Psychologist	11 (22%)	8 (16%)	0.46
Support worker	5 (10%)	9 (18%)	0.25
Other clinician	3 (6%)	1 (2%)	0.31

In summary, DLB patients attended a higher number of appointments than non-DLB patients over the course of their contact with secondary care. DLB cases seen by a specialist nurse attended a higher number of appointments than non-DLB cases, both overall and in the time before dementia diagnosis. They also saw medical practitioners on a higher number of occasions than their non-DLB counterparts.

A higher proportion of DLB patients than non-DLB patients saw a social worker throughout their contact with services. In the time prior to diagnosis, a higher proportion of the DLB group had contact with a social worker, a specialist nurse or an occupational therapist.

5.3.5 DLB symptom prevalence

We have discussed how 39% of DLB patients in this sample were initially assigned an alternative dementia subtype, and how the time from both referral and first review to final diagnosis is longer in our DLB group than our non-DLB group. As the presence or absence of clinical symptoms plays an important part in DLB diagnosis, we investigated the frequency of core, suggestive and supportive features at initial dementia diagnosis, final dementia diagnosis, and at the point of data collection.

The frequency of most core, suggestive and supportive symptoms increased over the course of DLB patients' contact with services, from first assessment to initial dementia diagnosis (mean 251

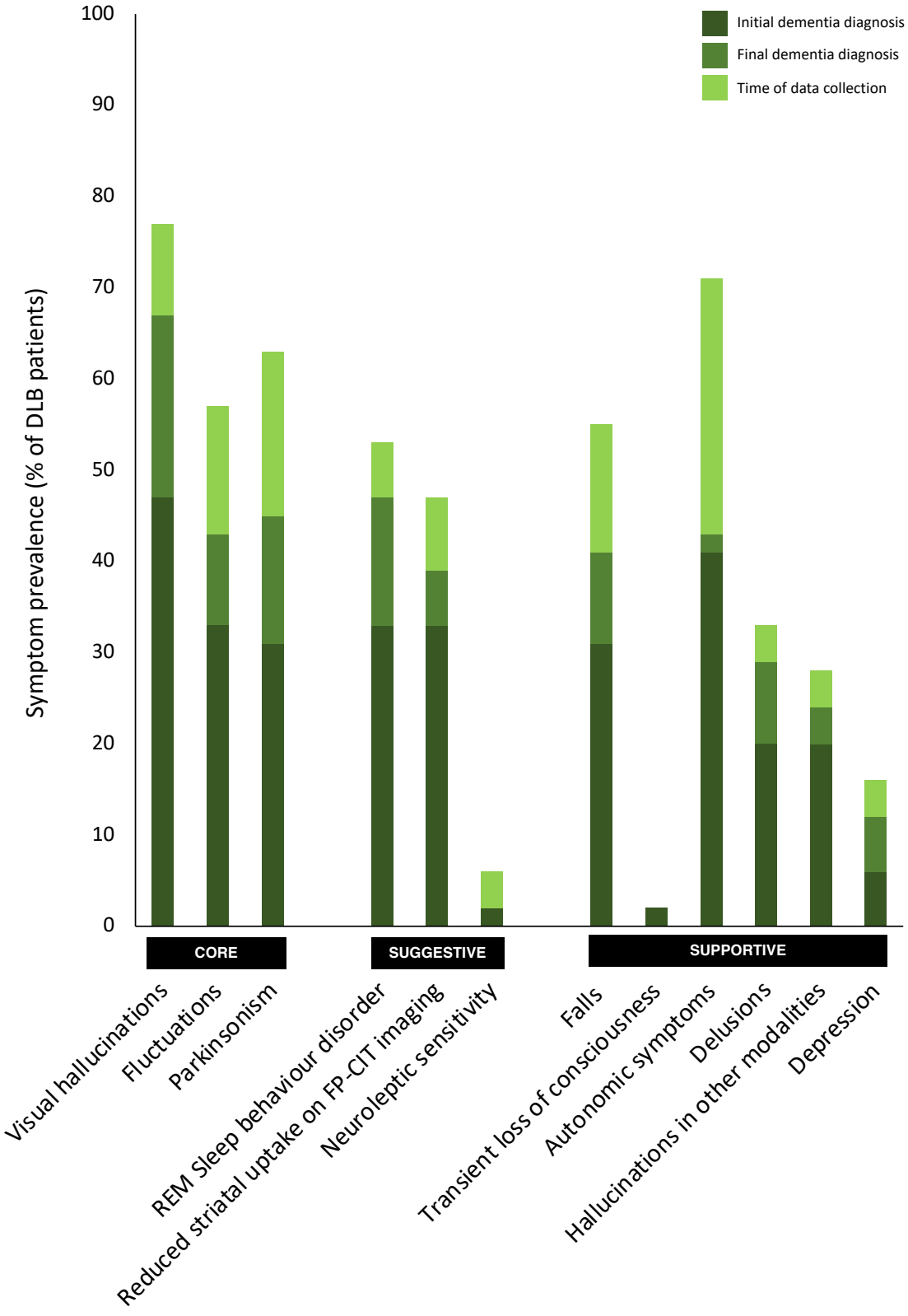
days, SD ± 811), to final diagnosis (481 days ± 623), to the point of our final data collection (1365 days ± 811) *(Table 5.19. Figure 5.4).*

Twenty-four participants (24/51; 47%) had had an abnormal FP-CIT scans at the point of data collection. This includes three patients who had two FP-CIT scans during their contact with services; in each case the first of these scans were reported as normal, but the second scan reported abnormal findings. Six of the twenty patients in our DLB cohort initially diagnosed with another dementia subtype had abnormal FP-CIT scans between their initial diagnosis and final DLB diagnosis.

Table 5.19 Prevalence of symptoms at initial dementia diagnosis, final diagnosis and data collection

	At Initial dementia diagnosis n (% DLB patients)	**At final dementia diagnosis** n (% DLB patients)	**At time of final data collection** n (% DLB patients)
Visual Hallucinations	24 (47%)	34 (67%)	39 (77%)
Fluctuations	17 (33%)	22 (43%)	29 (57%)
Parkinsonism	16 (31%)	23 (45%)	32 (63%)
REM Sleep behaviour disorder	17 (33%)	24 (47%)	27 (53%)
Reduced striatal uptake on FP-CIT imaging	17 (33%)	20 (39%)	24 (47%)
Neuroleptic sensitivity	1 (2%)	1 (2%)	3 (6%)
Falls	16 (31%)	21 (41%)	28 (55%)
Transient loss of consciousness	1 (2%)	1 (2%)	1 (2%)
Autonomic symptoms	21 (41%)	22 (43%)	36 (71%)
Delusions	10 (20%)	15 (29%)	17 (33%)
Hallucinations in other modalities	10 (20%)	12 (24%)	14 (28%)
Depression	6 (12%)	9 (18%)	11 (22%)

Figure 5.4 *Prevalence of symptoms at initial dementia diagnosis, final diagnosis and data collection (DLB patients)*



5.4 Summary

We conducted this study in two phases; the first, a retrospective observational study of DLB prevalence in clinical services; the second, a detailed observational analysis of patients' interactions with secondary care services and their observed clinical symptoms over the course of these interactions.

From the first phase, we found that DLB was diagnosed in 5.6% of cases of dementia in secondary care, with non-significant variation between participating services. DLB was significantly higher amongst men and younger patients.

Fifty-one of 144 DLB cases identified during the first phase were recruited to the second phase, in which information on the time, frequency and nature of interactions with secondary services was retrieved and analysed. Participants with DLB were initially given an alternative subtype dementia diagnosis, prior to subsequent diagnostic revision, in 39% of cases.

The duration of time from referral to final diagnosis was longer for patients with DLB than for those with non-DLB dementias. DLB was associated with longer periods of time from first assessment to final dementia diagnosis, and from initial dementia diagnosis to final dementia diagnosis. Cases with DLB who had FP-CIT imaging experienced longer periods between referral and initial assessment, and referral to initial dementia diagnosis, but not between referral and final dementia diagnosis. No relationship was observed between FP-CIT scan and proportion of DLB patients requiring diagnostic revision during the course of contact with services.

All prediagnostic and post-diagnostic clinical contacts with secondary services were recorded. Patients with DLB attended a higher number of appointments than those with non-DLB diagnoses over the course of their contact with secondary care and recorded a higher number of contacts with specialist nurses and medical practitioners over this time. DLB subjects also had a higher frequency of contacts with specialist nurses than non-DLB subjects in the time prior to dementia diagnosis.

A significantly higher proportion of patients with DLB saw a geriatrician or social worker during the entirety of their contact with services than those with non-DLB diagnoses. Although a higher proportion of patients with DLB than non-DLB dementias were seen by a social worker or

occupational therapist prior to dementia diagnosis, a greater proportion of non-DLB patients were seen by a specialist nurse in this period.

Our review of clinical symptoms in DLB patients observed that the prevalence of core, suggestive and supportive symptoms increased over time. Visual hallucinations was the most frequently recorded core symptom at each of three recorded time points.

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Chapter 6

A study of the frequency of DLB cases in NHS clinical services - discussion

6.1 Introduction

We conducted a longitudinal observational cohort survey of three Psychiatry of Old Age (POA) services in North East England, recording the age, gender, MMSE and clinical diagnosis of the 2575 patients attending these services over an 18-month period. We performed a detailed retrospective notes review of 51 patients with DLB and 51 with non-DLB dementia, comparing diagnostic practices, symptom prevalence and contact with services from the point of referral.

6.2 Prevalence and incidence of DLB

We identified that DLB formed 5.6% (95% CI 4.7-6.5%) of dementia cases. DLB was more common in male patients, and there was no significant difference in the age of DLB and non-DLB cohorts. We did, however find that DLB was found at lower rates within older age groups presenting to services. Our reported prevalence is lower than the 15-20% reported by neuropathological studies (Perry *et al.*, 1990), the 7.47% reported by meta-analysis of clinical samples (Vann Jones and O'Brien, 2014), and the 10.1% described by a recent epidemiological survey amongst a Chinese population (Yue *et al.*, 2016). Of newly diagnosed (incident) cases during the study period, DLB comprised a remarkably similar proportion of dementia cases (5.8%) to that seen in the prevalent population, though this fell well below the 25-28% reported from a large-scale Italian cohort (25-28%) (Bonanni *et al.*, 2013).

We will consider the factors that could contribute to the differences between our observations and those presented in previous studies; variation in true DLB prevalence, differences in study methodologies, and disparities in DLB detection. In doing so, we will compare our findings with some observations from our sister study, conducted at The University of Cambridge, that might suggest that differences in DLB case detection are more likely to be attributable to differences in DLB detection than variation in the true prevalence of the disease.

6.2.1 Neuropathological prevalence studies

It is possible that the difference between our observed DLB prevalence of 5.6% and the higher rates reported by other studies (Perry *et al.*, 1990) reflects a true variation in the prevalence of

pathology in dementia patients. If so, this could result from geographical differences in genetic burden or exposure to any environmental factors which may increase risk of DLB (Greenamyre and Hastings, 2004).

An important argument against the true DLB rates actually being lower in our cohort is the substantial body of evidence suggesting that cases demonstrating *post-mortem* DLB neuropathology frequently fail to meet clinical DLB diagnosis prior to death. High rates of revision of diagnosis, both over the course of illness and at *post-mortem*, have been demonstrated in several studies (Perry *et al.*, 1990; Thomas *et al.*, 2017b). This would suggest that diagnostic inaccuracy does frequently occur, and that it is a significant factor in variation between samples and services. Additionally, studies have demonstrated that the sensitivity of third consensus criteria is both variable and suboptimal (McKeith *et al.*, 2000b; Aarsland *et al.*, 2008; Skogseth *et al.*, 2017).

As discussed in Chapter 2, it is likely that neuropathological studies provide us with an upper margin for true DLB prevalence. *Post-mortem* examination remains the most robust method of DLB diagnosis and the practice follows a structure outlined by the third consensus criteria (McKeith *et al.*, 2005). A high frequency of cases with mixed DLB/AD pathology are observed in autopsy samples (Ince, 2001; Jellinger and Attems, 2011) and the close relationship between tau progression and cognitive decline (Jellinger and Attems, 2008), have led to the suggestion that LB and LN pathology could reflect a non-specific end stage of AD (Skogseth *et al.*, 2017). As neuropathological studies include an older group of patients than those seen in clinical studies (Zaccai *et al.*, 2015), DLB would be expected to be observed at higher rates in the former than in the latter.

Where cases with mixed DLB/AD do present to clinical services, a high proportion will not demonstrate characteristic DLB features (Tiraboschi *et al.*, 2006; Thomas *et al.*, 2018b). This leaves us uncertain as to the clinical characteristics of patients with mixed pathology, and thus the clinical prevalence of pure DLB which potentially could be clinically identified. Comparison of neuropathological findings with data from studies such as ours is therefore of limited value from the perspective of clinical practice.

Interpretation of neuropathological data must also be made after consideration of the effect of selection bias. Subjects consenting to autopsy are more likely to be Caucasian, more highly

educated, and have a longer duration of symptoms than patients in clinical populations (Fillenbaum *et al.*, 1996; Tsuang *et al.*, 2006). Research centres conducting *post-mortem* studies overestimate pathologies like DLB due to their proximity to specialist clinics, and referral to such services are more closely related to severity of symptoms than the subtype diagnosis itself (Zaccai, Ince and Brayne, 2006).

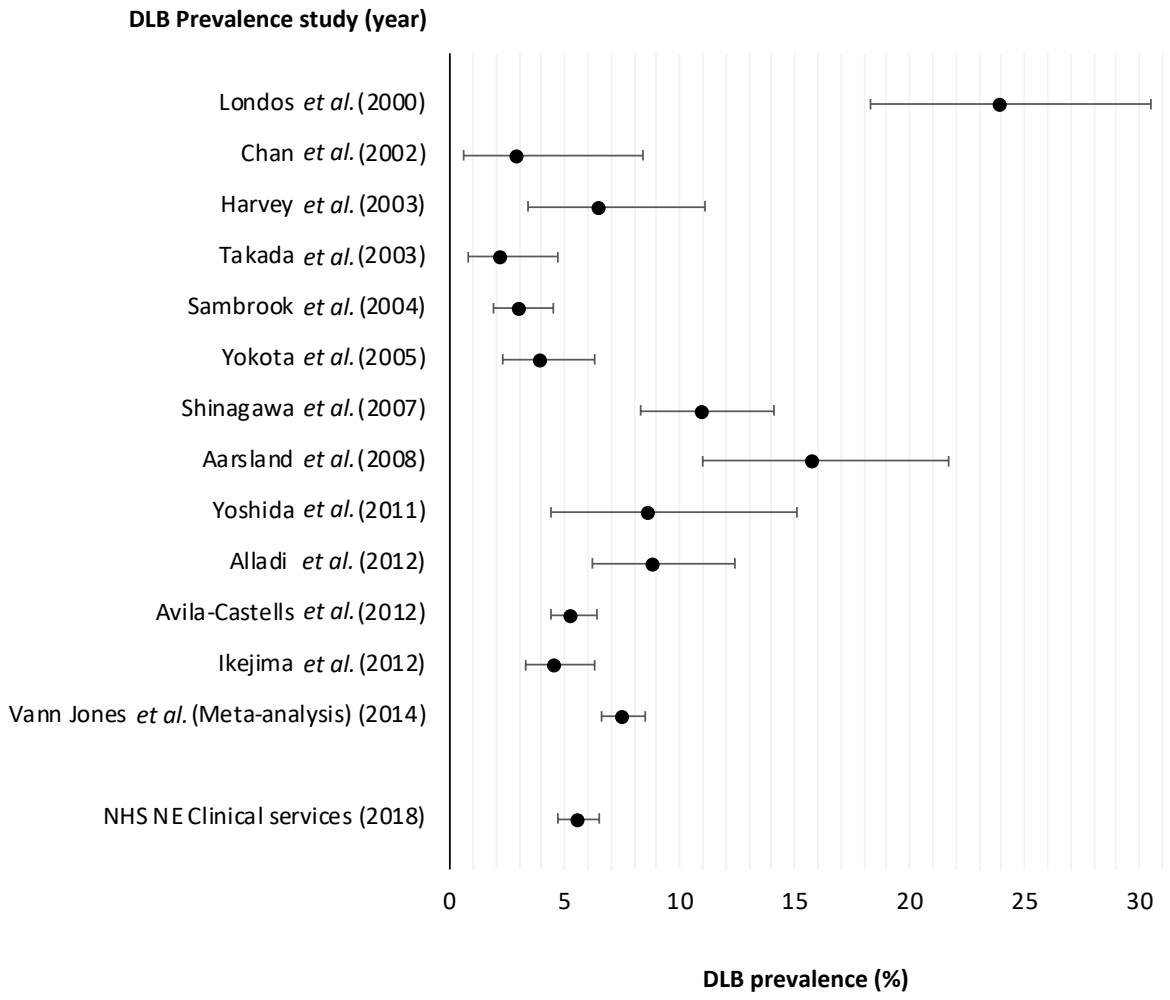
Post-mortem studies aid our understanding of the prevalence of DLB neuropathology, but the absence of core clinical features in a proportion of these patients dissuade comparison with clinical prevalence data and present a clear rationale for retaining a clinical perspective in examination of the factors influencing DLB diagnosis.

6.2.2 The role of methodology in DLB prevalence studies

The difference in DLB rates between our study and others might be due to differences in methodology used. Our cohort of 2 575 dementia patients is considerably larger than the ten clinic-based prevalence studies contributing to meta-analysis (sample sizes 102 to 766) and approaches the cumulative sample size of 3 144 (Vann Jones and O'Brien, 2014)(*Figure 6.1*). These smaller sample sizes have produced comparatively wide 95% confidence intervals, and of the six studies reporting higher DLB prevalence rates than those observed in our study, two have a lower confidence limit than both our reported prevalence (5.6%) and our lower confidence limit (4.7%) (Harvey, Skelton-Robinson and Rossor, 2003; Yoshida *et al.*, 2011).

Several studies contributing to meta-analysis have applied exclusion criteria that lower the denominator in any prevalence calculation and may contribute towards a selection bias. For example, some have excluded patients over 65 years old (Harvey, Skelton-Robinson and Rossor, 2003), moderate and severe dementia cases (Aarsland *et al.*, 2008), patients with a history of bipolar disorder or psychotic disorder, or those with a dementia subtype diagnoses other than AD, VaD, DLB or FTD (Yoshida *et al.*, 2011). Two studies were conducted in tertiary or specialist services, and thus likely to comprise a higher proportion of non-AD cases than in the secondary services contributing to our study (Shinagawa *et al.*, 2007; Alladi *et al.*, 2011). The highest rate of DLB in studies contributing to meta-analysis (24.0%; 95% CI 18.26-30.53) was reported by a group who drew their clinical sample retrospectively from a neuropathological cohort, reviewing *antemortem* patient notes to arrive a clinical diagnosis (Londos *et al.*, 2000).

Figure 6.1 Reported DLB prevalence rates among selected clinical studies



Of studies published since the aforementioned meta-analysis, an Italian study is of note as an outlier, reporting DLB incidence rates of 25-28% in a range of secondary and tertiary services (Bonanni *et al.*, 2013). Whilst such high figures are not unprecedented, they have not been reported by other studies of such scale and methodology. Neurology services contributing towards the data outnumber both geriatric medicine and POA services, although this is to be expected as neurologists diagnose the majority of dementia cases in Italy (Wilkinson, 2004; Azermai *et al.*, 2013). The study utilised a large cohort (2 042 patients) and adopted follow-up and diagnostic revision where necessary within the methodology. DLB prevalence was largely uniform across different specialities and between secondary and tertiary services, despite the lack of access to FP-CIT imaging in the former. Neuropathological validation data is not available, but in a sample this size, such measures are impracticable, and as discussed previously, subject to their own biases.

It is unclear whether the results of this study represent a higher prevalence of DLB in central Italy, a higher sensitivity to DLB amongst enrolled services, or the presence of other factors. Walker *et al.*(2015b) noted the higher observed prevalence rates in studies that included neurological examination in their methodology, but this alone is insufficient to explain such high figures for DLB incidence. There is perhaps a suggestion that incidence of dementia observed in the studied region is much lower than would be expected in an equivalent UK population (Matthews *et al.*, 2016) and the high rates of VaD also suggest that typical AD cases might be seen and diagnosed in other services (such as general practice) or are simply not presenting to services at all.

All but one of the aforementioned studies (Harvey, Skelton-Robinson and Rossor, 2003) were conducted in countries other than the UK. It is, of course, possible that DLB rates vary from country to country, but the variation in methodology and reported prevalence makes it impossible to draw any such conclusions on the strength of the data from our study. Nevertheless, the fact that we observed a range in DLB prevalence between individual services in North East England suggests that differences in true disease variation are not interregional. Indeed, our 3 services were very geographically close (16-33 miles apart), minimising the likelihood that genetic or environmental variation is a likely cause of the differences in prevalence.

The likely presence of such variation between services can be further supported by data from our sister study, conducted in East Anglia (Kane *et al.*, 2018). Our colleagues from The University of Cambridge adopted the same methodology, allowing us to scrutinise differences in epidemiology independent of aspects of study design. The team screened a patient population of 1 929 over six services (comparative in size to our three services) and reported a prevalence of 3.3% (95% CI 2.6 - 4.2%) and incidence of 3.8% (95% CI 2.8 -4.9%). Interestingly, prevalence rates within services contributing to this cohort ranged from 2.4% to 5.8%. This supports the hypothesis that observed differences in prevalence go beyond those attributable to methodology and compels us to examine other factors for this observed variation.

A range of methodologies have been used by DLB prevalence studies, but this heterogeneity has made comparison of these studies difficult. In demonstrating a range of DLB prevalence rates among participating services, using the same methodology, our findings provide support to the

suggestion that variation in DLB prevalence is primarily related to elements other than environmental factors.

6.2.3 The role of clinical factors in DLB prevalence

One such factor contributing to the range in DLB prevalence rates may be differences in rates of DLB detection. The observational nature of our study brings into focus the importance of service and clinician factors in DLB diagnosis. Other studies investigating DLB prevalence have adopted a selective, research-focused, highly detailed approach to case identification that often included comprehensive neurological examination, routine functional neuroimaging and the use of specialist rating tools on every patient within their respective cohort. Our methodology, deliberately designed to answer the clinically relevant question of current frequency of DLB diagnosis, recognised that several facets potentially contribute to case recognition outside the specialist setting, and that routine clinical practice may preclude such detailed approaches to diagnosis in every patient.

Thus, our real world and clinically representative sample included all patients attending participating services over an 18-month period; patients contributing to the data in the first phase of the study were not subject to any other inclusion or exclusion criteria. This allowed the process of diagnosis in clinical services to be viewed in a naturalistic NHS setting. Rather than a systematic process using validated scales, designed with the purpose of identifying DLB, clinicians working in contributing services had a broader, more pragmatic brief, subject to comorbidities, family dynamics and time pressures.

Other aspects of DLB diagnosis could adversely influence detection in clinical practice. The first is the suboptimal sensitivity of DLB criteria itself (Aarsland *et al.*, 2008); even if we were to assume that each clinician were to have judiciously applied consensus criteria to each case, at each appointment, a large proportion of cases will be not be detected. Thomas *et al.* (2017a) recently reported a sensitivity of clinical diagnosis of 87% against neuropathological diagnosis, highlighting that even expert clinical assessment is not sufficient to recognise all DLB cases in a population.

What is more likely is that a proportion of clinicians either fail to detect the core and suggestive features that comprise the criteria or fail to combine this information appropriately in formulating a diagnosis. This is complicated by the apparent masking of core DLB symptoms in cases with

concomitant AD pathology; a higher prevalence of visual hallucinations (33%) is reported in cases with low levels of AD pathology, than those with a higher burden of disease (65%) (Merdes *et al.*, 2003) and DLB detection rates are inversely correlated with Braak AD staging. Sensitivity for *antemortem* DLB is has been reported to be as low as 15% in cases demonstrating higher stages (Braak V-VI) of AD pathology (Weisman *et al.*, 2007).

The most likely source of variation between services is related to the structures of the organisations involved and the diagnosticians that comprise them. As an observational study, our data must be viewed through the prism of the organisations from which the data was retrieved; every point of the patient's journey, from initial presentation to his or her GP presents a factor that might impact upon overall prevalence and incidence within our sampled services.

An example of this is the fact that our study included only POA services. Should a patient present to their GP with DLB symptoms, and the GP decide that referral is indeed necessary, he or she may wish to refer to a service other than POA; autonomic dysfunction or falls may prompt referral to a geriatrician, or the presence of parkinsonism might increase likelihood to refer to neurology, even in the presence of cognitive dysfunction. In both of these types of services, non-DLB dementia is likely to be much less common, and therefore likely to raise our prevalence and incidence were they to be included in our sample population. Although such patients are often later transferred to POA *(Table 5.15)*, it is possible that younger, higher functioning patients requiring fewer social care or non-pharmacological interventions may be retained in, or discharged directly from, neurology or geriatric medicine services.

It is also entirely possible that there exist patients with clinical dementia within the general population that never come to the attention of any secondary care service. One meta-analysis has reported that 43% of dementia cases in UK populations go undetected (Lang *et al.*, 2017). The same study found that underdetection was associated with male patients and younger patients, two groups among which our study has observed higher prevalence rates of DLB.

It therefore appears likely that clinical factors, and in particular DLB case detection, could play an important role in clinical prevalence. The range of DLB case frequency among different services participating in the study (3.5% to 5.9%), and among those participating in our sister study in East

Anglia (2.8-5.1%) (Kane *et al.*, 2018), could therefore represent varying rates of clinical detection, as it is less likely that geographical differences in genetic or environmental factors could be present in neighbouring services. There may be a higher sensitivity for DLB amongst clinicians in participating North East England services, perhaps related to the prominent role of Newcastle University in the DLB research community and might explain the significant difference in observed prevalence between our cohort (5.6%) and those seen in the East Anglia cohort (3.3%). The prospect of accessing specialist support groups and experimental treatments through research participation may further incentivize clinicians to recognize the signs and symptoms of DLB.

In summary, there is evidence to suggest that the DLB prevalence rates we observed, and the range observed between different services, could be influenced more by clinical detection rather than variation in true disease prevalence. This is particularly the case when the findings of our sister study, also conducted in a UK clinical cohort, are considered alongside our data.

6.2.4 The relationship between DLB prevalence and DLB incidence

The relationship between our observed prevalence and incidence figures is important as few studies explore both of these parameters, citing either new cases presenting to services or prevalent cases in small, defined populations. The striking similarity between our figures for prevalence and incidence suggests that patients leave our services, either through discharge or death, at roughly the same rate as they enter. This may reflect pressures on services to discharge patients shortly after diagnosis and establishment of acetylcholinesterase inhibitor therapy but is somewhat surprising given the carer stress (Lee *et al.*, 2013) and higher rate of hospitalisation and neuropsychiatric symptoms (Hanyu *et al.*, 2009) that may necessitate longer follow-up in patients with DLB. This may be offset by the higher mortality associated with DLB compared with other dementias (Williams *et al.*, 2006; Price *et al.*, 2017).

6.2.5 DLB prevalence and gender

Our observation of a male preponderance of DLB is noteworthy in light of the findings reported by meta-analysis (Vann Jones and O'Brien, 2014), which did not find a difference in gender prevalence. The predominance of females was noted in five of eight included clinical studies, although this may be more closely linked to a failure to report gender composition in some studies than a composition of the samples themselves. A male preponderance observed amongst PD patients (Mayeux *et al.*, 1995) and neuropathological DLB samples (Klatka, Louis and Schiffer, 1996) has led to unsubstantiated suspicions that similar patterns of disease exist in clinical DLB populations. Subsequent population samples have both supported and refuted this hypothesis (Savica *et al.*, 2013; Yue *et al.*, 2016), but our data represents the strongest support for a male preponderance of DLB in a clinical sample to date.

6.2.6 DLB prevalence and age

We found no significant difference in age between our DLB and non-DLB cohorts. A positive relationship between DLB prevalence and mean age of sample has been observed in population-based studies, and but no such relationship has been identified in clinical populations (Vann Jones and O'Brien, 2014).

We did identify a separate age effect in observing that DLB comprised a higher proportion of dementia cases in younger age groups than in older ones. This observation that DLB prevalence decreased with age could be related to the fact we researched patients in clinical services; in such services, increasing age may be accompanied by a shift of clinical focus away from investigation and subtype diagnosis to a more pragmatic, needs-based service that aims to establish adequate social care. In some instances, older cases could circumvent POA services, and thus our study population, entirely; a patient with functional impairment due to physical illness, subsequently admitted to a nursing home to address this impairment, may not have cognitive symptoms identified as readily as a younger, less impaired counterpart, and would perhaps be less likely to be referred to a memory clinic. The higher mortality rate associated with DLB than AD (Price *et al.*, 2017), and thus higher representation of older patients with non-DLB dementias, is also likely to contribute to this finding.

The absence of an association between DLB and age in our overall sample, but a separate significant inverse relationship between increasing age groups and the DLB prevalence, might be explained by the relatively small number of patients that comprise groups at each end of the age scale. Three age groups representing patients with dementia 70 years old and under, 91 to 95, and 95 years and over comprised 7.8%, 8.7% and 1.9% (cumulatively 18.4%) of our sample, and DLB prevalence ranged between 3.9% and 8.0% within these groups. In four groups spanning the ages of 71 to 90, representing 81.6% of the overall sample, there was relatively little variation in DLB prevalence (4.8% to 6.2% of dementia cases). Whilst this suggest that DLB may not be associated with younger age groups, it could still be important for clinicians to retain a higher index of suspicion for DLB in the relatively small proportion of patients who present to services under the age of 70 years old than in other populations.

6.3 Time taken to DLB diagnosis

We found that the median time from referral to final diagnosis was 265 days for DLB cases and 125 days for non-DLB subjects. In many instances the delay involved reassessment; over one in three (39%) DLB cases were initially given an alternative dementia subtype diagnosis prior to subsequent diagnostic revision. In contrast, only one of 51 patients (2%) with non-DLB dementias had their dementia subtype diagnosis revised during their contact with secondary care.

Despite a recognition that differentiating DLB from AD may be challenging in clinical practice (Huang and Halliday, 2013), data on delayed diagnosis and revised diagnosis is sparse. A survey of carers of patients with DLB or PDD reported that 49% of diagnoses occurred over a year after initial presentation, with 31% taking over two years; only 9% received a diagnosis at first presentation (Galvin *et al.*, 2010). The same study reported that patients were given an initial diagnosis other than Lewy body dementia in 78% of cases. These figures may however reflect some of the differences characteristic of the USA healthcare system, and as survey findings may be affected by recall bias. Initial diagnosis appears to have occurred at the first point of contact with any service; whilst this not an uncommon practice in the UK, the median of 88 days between initial assessment and first non-DLB dementia diagnosis would suggest that it is not the convention in our participating services.

It is far from unusual for UK patients to undergo two or three clinical appointments, often with allied health professionals, prior to an initial diagnostic appointment. Clinicians working in

services contributing to our study not only had the benefit of prior assessment and neuropsychological testing, but in 33% of cases, abnormal FP-CIT results. This less immediate approach to diagnosis may explain the much lower rate of initial misdiagnosis in our cohort, but likely contributes to a longer period of time before a diagnosis is made; patients who had FP-CIT experienced a median of 103 days between initial assessment and initial dementia diagnosis, and 154 days between initial assessment and final diagnosis.

Our findings regarding time to diagnosis in the context of FP-CIT provide an insight into how the biomarker influences diagnostic practice. DLB cases who had FP-CIT imaging encountered a significantly longer period of time from referral to initial dementia diagnosis (median 210 days) than DLB cases who weren't scanned (100 days), perhaps suggesting that in such instances clinicians postpone subtype diagnosis until FP-CIT scans have been reported. However, patients who had FP-CIT scans did not encounter a significantly longer time from referral to final diagnosis (245 days) when compared with patients who did not have scans (358 days). This may be partly due to an observed non-significant trend for non-FP-CIT DLB patients to encounter a longer period of time for revision of diagnosis to occur than in FP-CIT DLB cases.

While this might be seen to suggest that FP-CIT does not shorten the period to DLB diagnosis, and prolongs the time to initial dementia diagnosis, it is important to consider that DLB patients referred for FP-CIT are likely to represent a different population from DLB patients that are not referred for scans. Clinicians are less likely to require the support of FP-CIT findings with more established disease, or a higher number of symptoms, that those with milder or fewer symptoms. One study suggested that clinicians referring patients with possible DLB for FP-CIT had a mean diagnostic certainty of 50% (Walker *et al.*, 2015a); this is likely to be higher in cases of probable DLB, with biomarkers adding little to clinical assessment. It may also be the case that some clinicians, anticipating the wait for initial dementia diagnosis associated with FP-CIT may be dissuaded from accessing the biomarker in some patients. Decreasing the waiting times associated with FP-CIT may therefore not only decrease the longer time to initial dementia diagnosis, but the proportion of clinicians accessing the investigation.

Although rates of diagnostic revision were equal in FP-CIT and non-FP-CIT DLB groups, the utility of FP-CIT as a biomarker for DLB is well established (McKeith *et al.*, 2007). It has demonstrated

superior sensitivity to expert clinical assessment when compared with autopsy findings (Thomas *et al.*, 2017a), and therefore could have the ability to detect DLB cases neither picked up by clinical assessment nor our diagnostic validation process. It also be noted that clinicians may use FP-CIT investigation not only in advising diagnosis, but perhaps in some cases in confirming diagnosis made on the basis of clinical symptoms alone; four (4/24; 17%) of the DLB patients who had FP-CIT in our sample had their scan after their DLB diagnosis had been made.

The period of time observed from referral to first assessment in DLB patients who had FP-CIT scans (median 47 days) compared to those who did not (median 22 days) may suggest that DLB cases with clearer clinical features are both less likely to require use of biomarkers and more likely to be seen more quickly in clinical services. Visual hallucinations, where identified, are often recognised as a "*red flag*" symptom in both GP and POA services, expediting initial assessment, and the symptom was the most prevalent core DLB feature identified in our cohort. Thus, clearer clinical symptoms could both have expedited assessment, and diminished the need for diagnostic investigations.

6.4 Contact with clinical services

We found that patients with DLB attended a significantly higher number of appointments during their contact with services (median 17 appointments) than those with other dementia subtypes (median 10 appointments). Although studies in both USA (Murman *et al.*, 2003; Zhu *et al.*, 2008) and Sweden (Boström and Jönsson, 2007) have reported higher resource use by DLB patients, only the Swedish group identified a significant difference in outpatient appointment contact, or the cost of this input. Our findings therefore provide an important insight into resource use by DLB patients in UK clinical practice.

DLB cases were seen by medical practitioners with a significantly higher frequency (median 7 appointments) than their non-DLB counterparts (5 appointments), perhaps reflecting the greater clinical complexity of DLB presentations. The higher rates of neuropsychiatric symptoms and inpatient admission in patients with DLB (Hanyu *et al.*, 2009; Mueller *et al.*, 2018) are likely to have required both management and repeated assessment. This may also explain why DLB patients attended a higher number of appointments with specialist nurses (median 11 appointments) than those with non-DLB dementia subtypes (3 appointments).

A higher proportion of DLB patients than non-DLB patients attended appointments with geriatricians during their contact with services (*Table 5.17*). Symptoms like parkinsonism, falls, and autonomic dysfunction, all associated with DLB, are likely to have prompted referral to geriatric services, either from POA practitioners or from other professionals. This relationship did not reach statistical significance when prediagnostic appointments alone were included in analysis, lending further support to the development of additional DLB symptoms over time.

We had decided against correcting this data for multiple comparisons on the basis of its exploratory and *post hoc* nature (Armstrong, 2014), but it could be argued that in comparing DLB and non-DLB appointments with up to ten allied healthcare professionals, that we should have corrected this accordingly. Were we to have used a Bonferroni-adjusted significance level of p=0.005, all of our non-corrected statistically significant findings related to appointments attended during the course of secondary care would have reached statistical significance; DLB patients attended a higher frequency of appointments with both medical practitioners and specialist nurses than their non-DLB controls. However, our observation that cases with DLB attended a higher number of prediagnostic appointments with social workers and lower number with specialist nurses than non-DLB patients would not have reached this corrected significance level. Caution should be exercised before dismissing these prediagnostic symptoms entirely, however, not least due to the conservative nature of the Bonferroni correction (Armstrong, 2014). Access to a larger sample size, perhaps by combining our data with that of our East Anglian sister study, could offer a further insight into the relationship between DLB and prediagnostic clinical contact.

A significantly higher proportion of patients with DLB than those with non-DLB dementia had contact with social workers both prior to diagnosis, and over the entire course of their contact with clinical services. A possible reason for this finding could be explained by the frequent role of social workers in the process preceding compulsory admission to hospital. Symptoms like delusions and hallucinations, as well as increased rates of carer stress (Bjoerke-Bertheussen *et al.*, 2012), are all frequent precipitants of inpatient admission in DLB, and the disease is associated with higher rates of hospitalisation (Hanyu *et al.*, 2009). It is possible that detention under mental health legislation, or consideration of detention, could have contributed to our observed figures. Another role of the social worker in NHS clinical services is identification of suitable residential and care home placement when it is no longer possible for patients to remain in their

own home, and the time from initial assessment to nursing home admission is significantly shorter in DLB patients when compared to AD patients (Rongve *et al.*, 2014). The fact that a significantly higher proportion of DLB patients had contact with an occupational therapist prior to diagnosis could support a higher degree of functional impairment than that seen in matched non-DLB dementia patients.

In both prediagnostic appointments, and in overall appointments, non-significant trends for DLB patients to be seen both in higher proportions, and with greater frequency than non-DLB patients, is observed amongst most professional groups. It is possible therefore that the number of patients recruited simply did not provide us with the power to adequately detect significant differences between the two groups, particularly given our finding that 39% of patients with DLB will undergo revision of diagnosis, which itself require additional clinical appointments, treatments and follow-up. Combination of our data with that of our East Anglian counterparts may help clarify this.

Our findings with respect to the higher proportion of DLB patients than non-DLB patients attending geriatric medicine services, and the higher frequency with which the former attend specialist nurses and medical professionals, strongly suggest that DLB is associated with higher healthcare costs than other dementia subtypes in a UK population. Indeed, unpublished data from our group, using the same cohort of participants, will report that the mean costs of secondary care for DLB subjects were £6,557 compared with £3,425 for matched non-DLB controls. It may be that opportunities to enhance the management of DLB in NHS services could be pursued in an effort to decrease the extent of contact required, but it may also support the allocation of greater resources to services routinely managing high rates of patients with the disease.

6.5 DLB symptom prevalence

We found that the proportion of DLB patients with a recorded history of core, supportive and suggestive features increased over the course of contact with services. Visual hallucinations was the most prevalent of the core symptoms, followed by parkinsonism. The increased proportion of patients demonstrating core and suggestive features over time suggest that services are continuing to detect DLB symptoms not only after initial diagnosis, but after revision of diagnosis has occurred.

6.5.1 Visual hallucinations

At each of the three time points observed, visual hallucinations were the most commonly recognised of all core, suggestive and supportive symptoms, present in 77% of DLB cases. This figure is towards the lower end of the 69-100% range observed in both population-based and clinical cohort studies (Aarsland *et al.*, 2008; Jefferis *et al.*, 2013; Savica *et al.*, 2013; Yue *et al.*, 2016). Visual hallucinations are an important symptom to detect. Tiraboschi *et al*. (2006), despite reporting a lower rate of the symptom at presentation (22%) than that seen at our point of initial dementia diagnosis (47%), found that visual hallucinations were a highly specific feature for differentiating DLB and AD in a neuropathologically-validated cohort.

It is important to recognise that our data relating to each of the observed clinical features is based on the simple presence or absence statements in clinical records. Visual hallucinations, however brief or poorly formed, were recorded as present for DLB patients, if documented in the clinical notes. This methodology therefore failed to capture the qualitative and phenomenological information necessary to differentiate complex and repeated visual hallucinations characteristic of DLB from the simple hallucinations associated with eye disease and those associated with other aetiologies, such as delirium. However, it could be expected that practitioners working in the POA setting, though less comfortable in assessing parkinsonian and RBD symptoms, would be more accustomed to a more phenomenological approach to detection and interpretation symptoms like visual hallucinations than their colleagues in other specialties.

6.5.2 Fluctuations in alertness and consciousness

Fluctuations in alertness and consciousness were detected in 57% in our DLB sample, and only 33% at the point of initial dementia diagnosis. Fluctuations have been suggested as occurring in over 80% of DLB patients (Byrne *et al.*, 1989), but even well-characterised research samples have reported the symptoms in proportions as low as 25% (Palmqvist *et al.*, 2008; Savica *et al.*, 2013; Walker *et al.*, 2016; Yue *et al.*, 2016). Detection of fluctuations in research samples is complicated by poor inter-rater reliability (Mega *et al.*, 1996; Luis *et al.*, 1999), and accurate observation is likely to be even more variable in clinical populations like ours. Although identification and characterisiation of fluctuations have been made easier by the Clinician Assessment of Fluctuation and the One Day Fluctuation Assessment scales (Walker *et al.*, 2000), in most cases these tools

have failed to have been adopted into clinical practice to the same degree as cognitive or neuropsychiatric scales.

The lower rate of fluctuations identified in our sample may not be solely attributable to clinical sensitivity for the symptom and may reflect the therapeutic effect of cholinesterase inhibitor prescription (Onofrj *et al.*, 2003). It is therefore possible that the widespread use of cholinesterase inhibitors in a clinical population not only diminished the observed prevalence of such symptoms, but in some cases prevented re-referral and after initial assessment and discharge from services.

6.5.3 Spontaneous parkinsonism

Parkinsonism was noted in 63% of our DLB cohort and was the core feature least frequently recognised at the point of initial dementia diagnosis (31%). The overall proportion of patients was considerably less than the 64 - 79% cited in some other studies (Harvey, Skelton-Robinson and Rossor, 2003; Savica *et al.*, 2013; Walker *et al.*, 2016); a comparable proportion of patients with tremor alone has been reported in a clinical DLB cohort (Onofrj *et al.*, 2013).

A lower prevalence of parkinsonism in comparison with other cohorts is not entirely unexpected; DLB patients participating in clinical trials are likely to be better characterised, and more systematically examined, with the use of scales such as the UPDRS, than those in observational studies such as ours (Savica *et al.*, 2013; Yue *et al.*, 2016). Our study design, incorporating only POA services, is likely reflecting a less sensitive approach to history and examination of parkinsonian symptoms, or in many cases, no neurological examination at all. This may, in part, be related to the perceived value of detecting parkinsonian symptoms; a recent survey of clinicians in tertiary DLB services suggested that only 61.8% of diagnosticians considered extrapyramidal signs to be relevant for DLB diagnosis, less than the proportions who considered visual hallucinations (94.1%) or fluctuating cognition (76.5%) relevant (Bonanni *et al.*, 2016)

Our detected prevalence of parkinsonian symptoms at diagnosis is somewhat comparable to the 25-50% cited in some areas of the literature (Walker *et al.*, 2016) but the higher rate of emergence of extrapyramidal symptoms reported by longitudinal studies (Ballard *et al.*, 2000; Walker *et al.*, 2015a) suggest that detection of such symptoms after initial diagnosis may be suboptimal in clinical services. This may suggest that a more systematised, longitudinal approach to examination of parkinsonism could feasibly be implemented in some services, leading to a higher case

detection and clinical DLB prevalence. Further support to this point is lent by the observation that of studies contributing to Vann-Jones and O'Brien's DLB prevalence meta-analysis (2014), those that included neurological examination in their methodology detected higher DLB prevalence rates than those that did not (Walker *et al.*, 2015b). One proposed mechanism for improvement of detection of parkinsonian symptoms is the routine use of an assessment toolkit for DLB (Thomas *et al.*, 2017b).

6.5.4 REM sleep behaviour disorder

RBD itself has been shown to have a high sensitivity for Lewy body pathology; 76% of DLB patients demonstrated PSG-validated RBD (Ferman *et al.*, 2011). The presence of RBD in patients with two other core DLB features increased the sensitivity of consensus to 88% (Ferman *et al.*, 2011). Although included as a suggestive feature of the third consensus criteria (McKeith *et al.*, 2005), RBD's sensitivity and early emergence relative to other symptoms has led to its classification as a core feature in the fourth consensus criteria (McKeith *et al.*, 2017).

Much smaller proportions (15-20%) of RBD in DLB are reported in epidemiological studies (Grace, Walker and McKeith, 2000b; Rongve, Boeve and Aarsland, 2010; Yue *et al.*, 2016), often in the context of high rates of sleep disturbance in dementia, with DLB patients more likely to suffer from disturbances than those with AD (Grace *et al.*, 2002; Farina *et al.*, 2009). Many of these studies employed validated questionnaires such as the Mayo Sleep Inventory, a component of which was reported as having 98% sensitivity for RBD (Boeve *et al.*, 2011).

Thus, caution should be applied in interpreting the 53% prevalence of RBD in our DLB cohort, none of whom had PSG studies. It is also unlikely that the Mayo Sleep Inventory was administered to any more than a handful of patients. In addition to the overall prevalence, the failure to determine any difference in RBD prevalence between our male and female cohorts lends further uncertainty to our findings, as the disorder has been shown to have a strong male preponderance.

The diagnostic utility of clinical history taking for RBD in our included services could therefore be legitimately questioned. Conflation of RBD with less specific sleep disorders could easily occur, especially in the form of confirmation bias; sleep symptoms may be more likely to be characterised as RBD in the presence of core symptoms or positive FP-CIT scanning. Further

research comparing clinical sensitivity and specificity of participating services against either validated questionnaire and/or PSG is necessary to accurately interpret our findings.

6.5.5 Neuroleptic sensitivity

Neuroleptic sensitivity was detected in three DLB cases (6%). This small sample size is very likely to be attributed to decreasing rates of antipsychotic prescription in patients with dementia in the UK over the last decade; antipsychotic medications were prescribed to 11.4% of patients with dementia in 2015, compared with 22.1% in 2005 (Donegan *et al.*, 2017).

Although increasingly rare due to the declining rate of prescription, neuroleptic sensitivity has been shown to be a specific sign for Lewy body disease, occurring in 30-40% of patients prescribed antipsychotics (Aarsland *et al.*, 2005). Varying practices therefore make comparison of observed neuroleptic sensitivity with that of other groups challenging, and statistics for prevalence of the symptom are not always provided. Figures of 6.7%- 10.3% have been reported in Chinese (Yue *et al.*, 2016) and Finnish community samples (Rahkonen *et al.*, 2003), but smaller, clinical samples often report no cases at all amongst smaller study clinical groups (Aarsland *et al.*, 2008).

6.5.6 Low dopamine transporter uptake in basal ganglia

An important factor in DLB diagnosis, and thus clinical prevalence, is likely to be the use of FP-CIT imaging as a biomarker. Although the utility of FP-CIT has long been established (McKeith *et al.*, 2007; O'Brien *et al.*, 2014b), Walker *et al*. (2016) demonstrated its usefulness in the clinical setting, frequently contributing to a change in diagnostic category and improving diagnostic confidence. The former is apparent in our DLB group, with three patients who had FP-CIT imaging demonstrating evidence of abnormal striatal uptake after initial dementia diagnosis, who subsequently had a revision of diagnosis to DLB.

The high proportion of patients in our sample who had abnormal FP-CIT imaging prior to initial dementia diagnosis (n=17, 33%) may go some way to explaining the high rate of DLB detection at initial diagnosis when compared with that of their American counterparts. Galvin *et al.* (2010) reported that patients were given an initial diagnosis other than DLB in 78% of cases, in a study conducted one year before the USA Food and Drug Administration approved the use of FP-CIT imaging in DLB diagnosis. It is also possible that the infrequent use of FP-CIT in the services surveyed by our sister study in East Anglia may contribute to their significantly lower rate of DLB

prevalence in the same area; only one (4%) of 23 patients that underwent detailed case note review had FP-CIT imaging, compared with 52% in our DLB group ($p < 0.01$).

In summary, we found that prevalence of core, suggestive and supportive features increased over the course of DLB patients' contact with services, including the use of the biomarker FP-CIT. Each of the clinical symptoms contributing to diagnosis were, however, observed at lower rates than those described by many research samples adopting a more systematic approach to symptom identification.

6.6 Study strengths and weaknesses

This study represents one of the largest cohorts to date examining DLB epidemiology, and the largest in a UK population. Through an observational study design, it lends clinical context to the existing data relating to neuropathological and clinical studies that report varying rates of DLB diagnosis. Similarly, our observations on symptom prevalence allow us to compare clinical data with the findings of more systematic studies that utilise validated questionnaires and expert assessment. Data derived from case note review is both detailed and longitudinal, allowing us to observe symptom prevalence and disease status at various points of the patient's journey before, during and after the diagnostic phase, and illustrating the disease course of DLB.

Our data relating to time to diagnosis and degree of clinical contact is largely unprecedented and gives us a unique insight into the patient and carer's experience in interacting with services. It makes a clear case to policy makers and service coordinators to allocate proportionate resources for the care of patients with DLB. We anticipate that a detailed economic analysis of clinical contact, conducted using in the same patients as our study, will report that the cost of care for patients with DLB is significantly higher than that of patients with non-DLB dementias.

Our study provides baseline data on DLB prevalence against which the effect of clinical interventions and tools, such as that proposed by Thomas *et al*. (2017b) can be measured. It also provides a yardstick against which the representativeness of smaller study cohorts could be measured.

Our study, nonetheless, is subject to limitations. The accuracy of the data collected used in this study is greatly determined by that of the notes reviewed, and the consultations which they detail. Bias could influence every point of the information gathering process from symptom to data collection. Patients and carers must have recognised a symptom in order to report it to clinician, provided that they recall having done so, for the clinician may or may not have asked about the symptom in question. The clinician must consider this symptom noteworthy to document it in the clinical notes or have deemed it sufficiently significant to communicate to the patient's GP, and therefore logged it during the data collection process.

The significance of each symptom itself could be argued to be related to the distress conferred by the symptom to the patient and carer. Although we found visual hallucinations and fluctuating attention to be the most prevalent and least prevalent of core DLB features respectively, this may in reality represent a reporting bias based how problematic these features are to patients and carers. Thus, patients developing fluctuations and RBD after a diagnosis of AD may not present again to health services, and diagnosis would therefore not be revisited; a patient developing visual hallucinations and neuroleptic sensitivity, however, could be much more likely to undergo revision of diagnosis.

The data to which the research team had access was restricted to the clinical records held by each participating organisation. This is relevant as two of the participating services formed part of a mental health trust, and therefore did not include complete data regarding patients' interactions with local acute trusts. Information from clinic letters and discharge summaries from acute care, filed in mental health notes as per standard practice, did provide us with some information. However, these communications usually represent the most condensed, immediately necessary clinical information, and it is likely that some data relevant to the study may not have been included in such documentation.

Every patient diagnosed with DLB during the Phase 1 screening period was approached for consent to participate in Phase 2. Nevertheless, a number of factors prohibited us from recruiting all identified DLB patients; the exclusion of patients since deceased, and those for whom approach was deemed inappropriate, could have contributed to a recruitment bias against patients with more severe disease. We also failed to adequately cross-reference databases from Phases 1 and 2. This meant that age and sex data (gathered during Phase 1) for patients who did not participate in

Phase 2 were not available for comparison with those of who did participate, and this precluded further exploration of potential recruitment bias.

Although our sample size was sufficient to detect differences between DLB and non-DLB cohorts, our subgroups were too small to determine differences between male and female patients, and differences between patients diagnosed with DLB at initial presentation or after diagnostic revision. The observed preponderance of DLB amongst men observed in Phase 1, and the high rate of DLB recognition at the point of presentation contributed to smaller sample sizes of the groups in question. In particular, the increased rate of observed visual hallucinations amongst female DLB patients, observed at all three time points, may have yielded more significant results with a larger sample size. Combining our data with that of our sister study in East Anglia may remedy this issue.

6.7 Conclusions

We observed that DLB represented 5.6% of dementia cases in clinical services, with DLB more common among men and younger patients. We observed a range in DLB prevalence (3.5-5.9%) among different clinical services, in keeping with data collected by our sister study and suggesting that variation in the DLB frequency could be related to clinical factors such as case identification. The time from referral to dementia subtype diagnosis was longer for DLB patients than non-DLB patients, and DLB patients required a larger number of clinical contacts than non-DLB patients during the course of their care. Core symptoms increased in prevalence over time, but core and suggestive symptoms were seen at lower than figures cited in literature to date.

Although the factors underpinning our findings are multifactorial, a more systematic approach to both symptom identification, and application of diagnostic criteria, may increase case detection and thus increase prevalence. Of core and suggestive features demonstrated by DLB patients in our cohort, impaired striatal uptake on FP-CIT imaging was second only to visual hallucinations in prevalence. Furthermore, several scans were conducted after initial diagnosis, likely contributing to diagnostic revision. Our findings therefore support the use of biomarkers in DLB diagnosis, both in the initial phase of assessment and in the reappraisal of cases in whom the disease is not apparent or detected. Identification of alternative or complementary biomarkers may further enhance diagnosis in the clinical setting.

One such biomarker may be the measurement of cardiac denervation, using MIBG cardiac scintigraphy, to detect DLB. Though not typically employed in routine clinical use in UK populations, pooled analysis of MIBG has cited a superior sensitivity (98%) and specificity for DLB (94%) to both FP-CIT and expert clinical assessment (Treglia and Cason, 2012). It has also recently been included as an indicative biomarker in the fourth consensus criteria (McKeith *et al.*, 2017). The suggestion that autonomic denervation, and thus abnormal MIBG uptake, occurs early in the course of DLB pathology (Orimo *et al.*, 2005), suggests that MIBG could detect DLB at earlier time point than FP-CIT. It should be noted, however, that none of the data contributing to the MIBG evidence base has been conducted among a clinically representative UK population, and caution is urged in translating MIBG utility from study populations to our clinical cohort.

Chapter 7

MIBG cardiac scintigraphy as a biomarker for DLB – aims, hypotheses and methods

7.1 Aims

The primary aim of this pilot study was to determine;

- the feasibility and utility of MIBG as a diagnostic biomarker in distinguishing DLB from AD;
- whether optimal HMR cut-offs derived from other, nearly all Japanese, populations were applicable to the UK population.

It aimed to do so in a cohort of patients representative of a UK population; this included patients with medical comorbidities and drug histories that included agents potentially capable of interacting with MIBG uptake. In addition, we included a pilot study into the utility of MIBG as a biomarker in probable and possible DLB patients with a normal FP-CIT scan.

7.2 Objectives and hypotheses

Our objective was to determine the diagnostic utility of MIBG in distinguishing DLB from AD. We hypothesised that;

- mean myocardial MIBG uptake, measured by the late HMR, would be significantly lower in the DLB group than the AD group;
- MIBG would demonstrate good sensitivity (>80%) and specificity (>90%) in differentiating DLB from AD;
- there would be some probable DLB patients with a normal FP-CIT scan who show evidence of impaired myocardial MIBG uptake, suggesting variation in DLB pathophysiology.

For each of these hypotheses, late HMR was used as the primary outcome. This is recommended in diagnostic studies, as late HMR reflects the active neuronal uptake of MIBG without passive components and maximises the ratio of specific to non-specific binding (Rascol and Schelosky, 2009). Our use of late HMR is in keeping with the majority of published research in the field (Treglia and Cason, 2012).

7.3 Methods

7.3.1 Ethical and regulatory approval

Ethical approval for this study was granted by Newcastle and North Tyneside NHS Research Ethics Committee (REC). Approval was also sought and granted by the sponsor (Newcastle upon Tyne Hospitals NHS Foundation Trust), research and development departments at participating trusts (Northumberland, Tyne and Wear NHS Foundation Trust, Northumbria Healthcare NHS Foundation Trust, Gateshead Health NHS Foundation Trust) and by the Administration of Radioactive Substances Advisory Committee (ARSAC).

7.3.2 Sample size

Sample size was calculated based on the main hypothesis that the mean HMR will be lower in the DLB group compared to AD group. We conducted an *a priori* independent samples t-test power calculation using the Minitab programme (v17). Mean HMR values and standard deviations (SD) for DLB and AD populations in a large multicentre MIBG study were; HMR (DLB) = 1.8 (SD ± 0.7); HMR (AD) = 2.8 (SD ± 0.70) (Yoshita *et al.*, 2015). However, in our population the SD may be larger, and a smaller difference would be of clinical importance.

Table 7.1 Sample sizes required at 80% power and 95% confidence to detect various differences in mean HMR between DLB and AD groups for different assumed pooled HMR standard deviations

Estimated difference	Estimated SD	Sample size (per group)
1	0.7	9
1	1	17
0.7	1	34

The required sample size *(Table 7.1)* is sensitive to HMR variability, which is not well known in a group of well-characterised subjects in the UK population. We therefore aimed to recruit between 10-30 subjects per group within the time constraints of this pilot study.

7.3.3 Subjects

Patients were of both sexes and over the age of 60 at the point of recruitment. Each participant had been diagnosed by local clinicians as having probable DLB, possible DLB or probable AD. Each diagnosis was later independently reviewed by members of the research team, according to third consensus criteria for DLB (McKeith *et al.*, 2005) or NIA-AA criteria for AD (McKhann *et al.*, 2011).

All participants had a reliable informant, who was in contact with the participant at least twice per week. Patients were identified through referral from the North East DeNDRoN (Dementias and Neurodegenerative Diseases Research Network) Case Register, or note screening of local Psychiatry of Old Age, geriatric medicine and neurology clinic caseloads. Regional neuroimaging databases were also screened for potential participants.

Exclusion criteria for the study were minimised, with the aim of recruiting a clinically representative population from the NHS. Nevertheless, some factors established as reducing MIBG uptake were incorporated into exclusion criteria.

Although a large number of frequently prescribed medications have been identified as capable of potentially decreasing MIBG uptake (Giammarile *et al.*, 2008), only the restriction of labetalol and tricyclic antidepressants has been recommended on the basis of strength of published studies (Jacobson and Travin, 2015). For this reason, patients prescribed labetalol or tricyclic antidepressants were excluded.

Subjects with a history of ischaemic heart disease and past history of myocardial infarction (MI) were included in the study. Although transmural denervation is observed immediately after MI, sympathetic neuronal regeneration can take place over several months after the event (Carrió *et al.*, 2010). To conservatively account for the effects of acute denervation, patients with a recent history (within 12 months) of MI were therefore excluded from recruitment.

Patients with cardiac pacemaker *in situ*, or those with symptoms of cardiac failure corresponding to Class II ("*Slight limitation of physical activity. Ordinary physical activity results in fatigue, palpitation, dyspnoea or angina pectoris*"), III or IV of the New York Heart Association Classification (Marvin, 1964), were also excluded from participation. For the purposes of patient safety, potential recruits with a history of contraindications to either FP-CIT or potassium iodate were also excluded.

Where potential participants had not already provided permission to be approached directly, patients were contacted by their treating clinician and subsequently by JK to answer any questions or issues. Written informed consent was provided by the participant. In cases where potential

participants did not demonstrate capacity to provide consent, a nominated consultee provided written consent on their behalf.

As part of the study we also aimed to recruit an additional cohort of patients. Each patient met clinical criteria for probable or possible DLB, and had recently had an FP-CIT, reported by standard clinical report as normal, conducted as part of routine secondary care. These patients were identified through screening of local neuroimaging databases. No such cohort has been previously investigated in this manner, and so this was exploratory. Both the Braak hypothesis of DLB pathogenesis (Braak *et al.*, 2003) and previous studies demonstrating disagreement between MIBG and FP-CIT (Tiraboschi *et al.*, 2016) suggest that some of these patients will demonstrate cardiac denervation, and thus low HMR.

This cohort was subject to the same exclusion and inclusion criteria as the participants described in the main study and underwent investigations using identical methodology. Nevertheless, as these patients comprised a population less representative of clinical practice (by definition due to their false negative FP-CIT) their results are analysed and discussed separately.

7.3.4 Assessment and diagnosis

All patients underwent detailed diagnostic assessment by JK. This comprised a review of clinical records, including structural imaging findings, routine dementia screening and bloods, psychiatric and medical history review and physical examination, as well as results of a battery of tests and scales relating to neuropsychological ability, functioning and disease-specific symptoms. These scales are outlined below.

The revised Addenbrooke's Cognitive Examination (ACE-R) is a 100-point scale administered to the patient, measuring cognitive functioning across five domains – attention and orientation, memory, verbal fluency, language and visuospatial function (Mioshi *et al.*, 2006). Embedded within the ACE-R are the 30 questions that comprise the Mini-Mental State Examination (MMSE), a shorter cognitive assessment tool in routine use in clinical practice (Folstein, Folstein and McHugh, 1975). The widely-used motor subscale of the Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz *et al.*, 2008) was used to identify and characterise participants' parkinsonian symptoms. The scale rates the severity of each of 33 symptoms, including bradykinesia, tremor, rigidity and postural

instability, on a scale of zero (symptom not present) to four (severe). It has been validated (Ramaker *et al.*, 2002) and is in widespread use in dementia studies.

Functioning was measured using the Bristol Activities of Daily Living Scale (BADLS) (Bucks *et al.*, 1996) and Lawton Instrumental Activities of Daily Living Scale (IADLS) (Lawton and Brody, 1969), both administered to each patient's primary carer. Both scales are widely validated and used routinely in dementia studies. The BADLS rates a patient's abilities to perform 20 tasks on a scale from zero (able to perform task independently or not applicable) to three (not able to perform task), with a maximum score of 60 representing a high level of functional impairment. IADLS scores patients from zero (unable to perform task) to one (able to perform) on eight tasks, with a maximum score of eight denoting independent functioning in all included activities.

The Neuropsychiatric Inventory (NPI) is a scale administered to the carer to determine the presence, severity and frequency of a range twelve neuropsychiatric symptoms: delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behaviour, night time behaviours and appetite disturbance (Cummings *et al.*, 1994). The scale also measures the level of distress to the carer from each symptom. The NPI subscale records the frequency and severity of all hallucinations but could be used in this study as a measure of visual hallucinations, as no subject described hallucinations in any other modalities.

The revised Dementia Cognitive Fluctuations Scale (DCFS-R), measures six symptoms recognised as capable of reliably differentiating DLB from AD; variation in function, daytime sleepiness, daytime lethargy and overall level of consciousness, disturbed arousal and disorganised speech (Ferman *et al.*, 2004; Lee *et al.*, 2014).

Depressive symptoms were measured using the Cornell Scale for Depression in Dementia (CSDD) (Alexopoulos *et al.*, 1988), a 19-item clinician-administered scale that uses information retrieved from both the patient and carer.

Clinical Dementia Rating (CDR) rates level of impairment, as determined by clinicians' global assessment, in six domains – memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. An overall score from zero to three is calculated by

an algorithm (*https://www.alz.washington.edu/cdrnacc.html*) (Morris, 1993); a score of 0.5 is consistent with MCI or dementia, and 1 to 3 with dementia.

Each diagnosis was reviewed by two experienced diagnosticians (JK and Alan Thomas, AT; Professor of Old Age Psychiatry). Where disagreement arose between the two raters, a third independent rater, John O'Brien (JOB, Professor of Old Age Psychiatry) provided a deciding opinion. If needed, cases were discussed by all three diagnosticians and a consensus reached. All cases underwent a repeat clinical assessment, including repeat neuropsychological testing, one year following initial assessment.

7.3.5 MIBG image acquisition

All MIBG scans were conducted within 12 months of FP-CIT scans and within 2 months of recruitment. All MIBG scans were conducted at Newcastle Royal Victoria infirmary, using Siemens Symbia T Series or Siemens Symbia Intevo systems.

Gemma Roberts (GR; senior medical physicist) calibrated both cameras prior to the study, using a planar cardiac MIBG phantoms. GR adopted the same design employed by Nakajima *et al*. (2012) for standardisation of HMR at Japanese centres prior to a multicentre trial (Yoshita *et al.*, 2015). This standardisation study converted HMR values measured at each of the centres in this multicentre study to a "standardised value" to correct for differences in camera and collimator. The correction factor was based on measurement at each centre with a standard phantom and comparison of the values obtained (Nakajima *et al.*, 2012). We participated in a European study, using the same phantom as in the Japanese study to determine a correction factor for our systems with assistance from the team involved in the original work (Verschure *et al.*, 2017)

Although one of the purposes of the study was to determine an optimal HMR cut-off value for a UK population, a HMR cut-off was calculated for the purposes of initial sensitivity and specificity analysis. Rather than converting all of our HMR values to "standardised" ones, we calculated what the threshold cited by Yoshida *et al*. (2015) (2.1 for both early and late images) would have been on our system. Our cameras used medium energy general purpose (MEGP) collimators, which tend to give higher HMRs as they are more effective at removing spurious counts due to septal penetration (Chen, Cao and Dilsizian, 2011; Inoue *et al.*, 2014). We therefore needed to apply a slightly higher cut-off to be equivalent to the Yoshida *et al*'s (2015) study, which were

standardised to typical low or medium energy collimators, and therefore tend to have slightly lower HMR ratios than our systems. The 2.1 HMR cut-off used in the multicentre study was equivalent to 2.24 and 2.25 for Series T and Intevo systems respectively when conversion factors were taken into consideration, and a 2.2 cut-off was therefore adopted for this study. Individual HMR values were calculated to one decimal point throughout analysis, in keeping with published data and reflecting the level of accuracy realistically achievable in the clinical setting. Mean HMR values were calculated to two decimal points.

Patients were asked to administer all medications as prescribed on the morning of the study. On the day of the scan, a detailed history of medication prescription and administration was retrieved. Upon arrival subjects were administered 170mg of potassium iodate. This agent protects the thyroid gland from any free radioactive iodine that may be associated with both MIBG and FP-CIT radiopharmaceuticals. One hour after potassium iodate administration, a single bolus of 111 MBq (3 mCi) of ^{123}I-MIBG and a single saline flush was delivered intravenously.

Early planar images in anterior view were obtained 20 mins following MIBG injection. Images were obtained using a dual headed gamma camera fitted with MEGP collimators. Patients were positioned with arms down in supine position. Images were acquired over 10 minutes and stored in a 128 x 128 matrix.

Late planar images and were obtained 240 minutes following injection. Where possible, patients were positioned with arms above their head for the purposes of SPECT imaging, although SPECT images were not analysed as part of this study. Images were acquired over 10 minutes and stored in a 128 x 128 matrix.

Subjects were provided with 170mg potassium iodate and instructed to administer 12 hours following MIBG injection.

7.3.6 MIBG analysis

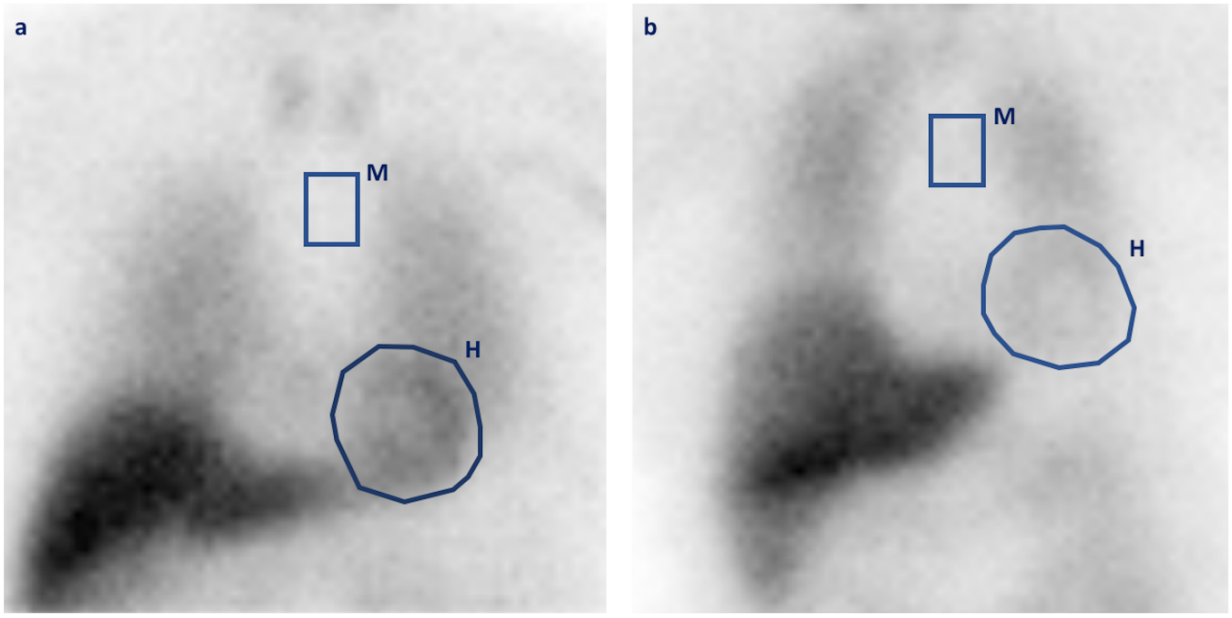
Regions of interest (ROIs) were identified for "*heart*" and "*mediastinum*". A myocardial region of interest ("heart" ROI), was circumscribed using freehand tool *(Figure 7.1).* A rectangular ROI of area 48 pixels was then placed on the midline of the upper chest, as a reference region of low and

consistent non-specific uptake. This size was chosen to be small enough to not be subject to interference from the lungs and large enough to give a reasonable sample of background counts.

ROIs were identified for both early and late images and HMR calculated in each case. This methodology is consistent with several previous studies exploring MIBG utility in DLB (Hanyu *et al.*, 2006; Yoshita *et al.*, 2006; Wada-Isoe *et al.*, 2007; Estorch *et al.*, 2008).

Figure 7.1 *MIBG Regions of Interest*

*Late anterior planar images of MIBG uptake in two study participants. Both depict 48-pixel mediastinal ROIs (M) and heart ROIs (H). Picture **a** demonstrates normal cardiac MIBG uptake (HMR 3.27); **b** demonstrates decreased uptake (HMR 1.46) suggestive of an abnormal scan result.*



Two raters, blinded to patient identity, measured the HMR on the early and late planar images independently (JK, GR). The mean of these two HMRs was calculated to two decimal points for use in further analysis and discussion.

Statistical analysis was performed using SPSS 24.0 for Windows. Continuous variables (including HMR) and categorical variables were analysed using Student's t test for independent samples and χ^2 test respectively. The Mann-Whitney test was used to compare the means of non-parametric data. We calculated sensitivity (true positives, the percentage where the HMR was ≤2.2 in patients with a clinical diagnosis of DLB), specificity (true negatives, the percentage where the HMR was

>2.2 in patients with a clinical diagnosis of AD), and accuracy (the percentage of times the HMR result matched the clinical diagnosis). Receiver operating characteristic (ROC) analysis was performed using the *pROC* package for the *R* statistical programme (version 3.3.1) (Robin *et al.*, 2011).

7.3.7 FP-CIT Image acquisition

FP-CIT imaging was conducted in accordance with established protocols (Thomas *et al.*, 2017a) at the Newcastle Royal Victoria Infirmary, Gateshead Queen Elizabeth Hospital or Sunderland Royal Hospital.

The study used data from FP-CIT scans conducted both as part of patients' routine clinical care, and those conducted within the study protocol. In cases where a participant had undergone FP-CIT imaging as part of their NHS care less than 12 months prior to MIBG imaging, this data was accessed and analysed, with permission, by the study team. Patients whom had previously undergone FP-CIT imaging more than 12 months prior to MIBG underwent a further scan at Newcastle Royal Victoria Infirmary.

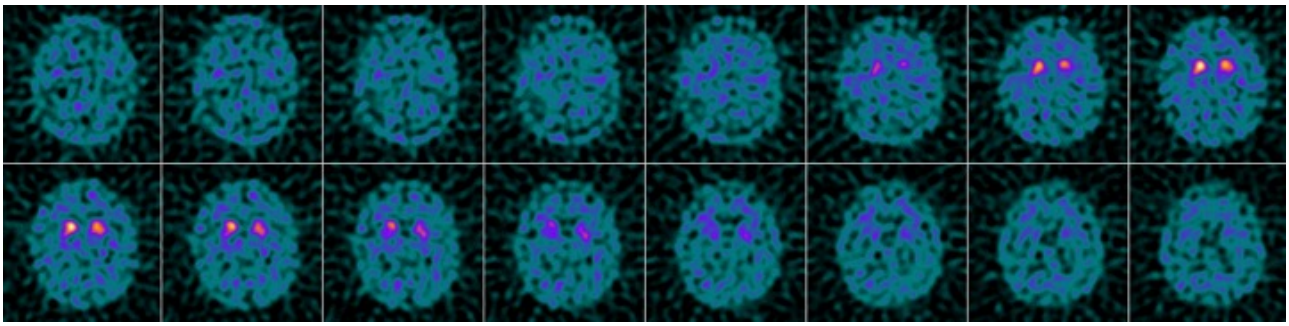
Patients were again asked to administer medication as prescribed and a record made of all medications on the morning of FP-CIT examination. One hour after administration of 170mg of potassium iodate, subjects received a single bolus injection of 111–185 MBq of ^{123}I-FP-CIT. Within 3–6 hours of FP-CIT injection, the patient underwent a brain SPECT scan to assess the functional integrity of the nigrostriatal dopaminergic neuron terminals in the striatum.

Image acquisition was conducted in accordance with standard FP-CIT practice, with a calibrated gamma camera fitted with a low-energy high-resolution (LEHR) collimator. Cameras were positioned prior to data acquisition, with as a radius of rotation as small as possible set without the cameras touching or distressing the patient. One hundred and twenty views in a circular orbit were acquired (3° steps over 360° rotation). The radius of rotation was set as small as possible. Acquired images were stored in a 128 x 128 matrix.
Transverse images were reconstructed using a ramp-filtered back projection with a Butterworth filter (cut-off 0.3 cycles/cm; order 10). They were not corrected for gamma ray attenuation. They

were converted to a DICOM format for subsequent visual and DaTQUANT analysis. For visual analysis 16 contiguous 1.95 mm slices were displayed using the "*GE*" colour scale *(Figure 7.2*).

Figure 7.2 FP-CIT Transverse images

Examples of the reconstructed transverse FP-CIT images reviewed by each rater. The images demonstrate significantly impaired reduction in bilateral putaminal FP-CIT uptake, with activity confined to the caudate nuclei, and therefore represents an abnormal study.



7.3.8 FP-CIT analysis

Transverse images of each FP-CIT scan were assessed by five independent raters blinded to patient identity and clinical information: one consultant medical radiologist, two consultant medical physicists experienced in nuclear medicine reporting, and two certified old age psychiatrists. Each completed a training exercise prior to assessing study images, comprising familiarisation with the visual rating scale and examinations of ten independent FP-CIT images ranging from normal to markedly abnormal.

Each transverse image was given a visual rating by each independent rater in accordance with established practice (Benamer *et al.*, 2000); 0 (normal uptake in all regions), 1 (asymmetric activity with one putamen showing reduced uptake), 2 (absent activity in the putamen of both hemispheres), or 3 (absent activity in the putamen of both hemispheres and greatly reduced in one or both caudate nuclei). Where three or more raters agreed upon a rating, this rating was used in further analysis. Where fewer than three raters agreed on a rating, the images were discussed at a meeting and a consensus reached.

JK used DaTQUANT software (provided by GE Healthcare) to calculate the specific binding ratio (SBR) of striatal uptake. The method provided an additional means of identifying abnormal scans that may have been rated as normal by visual rating (i.e. consensus score of 0).

The study design deliberately favoured visual rating over DaTQUANT result, in keeping with clinical practice and validation studies (Thomas *et al.*, 2017a). DaTQUANT was considered as an additional measure of sensitivity, rather than a means of superseding visual rating. Therefore, where any scans were identified as abnormal by DaTQUANT, but normal by visual rating, the panel were asked to reappraise and discuss the images in question. Where scans were identified as normal by DaTQUANT, but abnormal by visual rating, the visual rating was upheld.

The DaTQUANT quantification process followed established methodology (Tossici-Bolt *et al.*, 2006). Volumes of interest (VOIs) were semi-automatically placed over right and left putamina and caudate nuclei in the transaxial slice from which most intense tracer uptake was observed. JK repositioned striatal VOIs where required. Another VOI was placed over the occipital lobe to represent cortical background. SBR was calculated by dividing the mean counts per pixel in the VOIs corresponding to caudate nucleus, anterior and posterior putamen and the striatum as a whole, by the mean count per pixel in the occipital lobe VOIs. No attenuation correction measures were employed. Values for SBR were considered abnormal if they were more than two standard deviations below the normal population mean provided by the Parkinson Progression Marker Initiative (PPMI) database (Marek *et al.*, 2011).

Chapter 8

MIBG cardiac scintigraphy as a biomarker for DLB - results

8.1 Recruitment

We recruited 17 participants with DLB (16 meeting criteria for probable DLB, 1 for possible DLB) and 16 participants with probable AD.

Table 8.1 Demographic and clinical characteristics of DLB and AD participants

	DLB (n=17) Mean score (± SD)	**AD (n=16)** Mean score (± SD)	**p**
Age in years at MIBG scan (± SD)	77.0 (± 7.9)	76.4 (± 6.6)	0.81
Gender male/female (% Male)	15/2 (88%)	12/4 (75%)	0.33
Revised Addenbrooke's Cognitive Examination (ACE-R)	66.6 (± 15.1)	65.1 (± 15.7)	0.79
ACE-R subscale Attention and Orientation	13.9 (± 3.6)	13.7 (± 3.7)	0.89
ACE-R subscale Memory	13.4 (± 5.1)	11.4 (± 4.7)	0.24
ACE-R subscale Fluency	6.4 (± 3.3)	6.3 (± 3.4)	0.88
ACE-R subscale Language	22.3 (± 5.1)	21.8 (± 4.7)	0.78
ACE-R subscale Visuospatial	10.6 (± 4.1)	12.0 (± 3.3)	0.29
Mini-Mental State Examination (MMSE)	22.1 (± 4.9)	22.2 (± 4.2)	0.97
Bristol Activities of Daily Living Scale (BADLS)	22.3 (± 13.2)	13.9 (± 7.7)	**0.04**
Instrumental Activities of Daily Living Scale (IADLS)	2.6 (± 1.9)	3.7 (± 1.9)	0.13
MDS Unified Parkinson's Disease Rating Scale (UPDRS) Motor subscale	32.8 (± 23.4)	3.6 (± 3.6)	**< 0.01**
Neuropsychiatric Inventory (NPI) Hallucinations subscale (Frequency x Severity)	2.1 (± 2.5)	0.0 (± 0.0)	**< 0.01**
Dementia Cognitive Fluctuations Scale (DCFS-R)	10.9 (± 3.7)	6.5 (± 1.9)	**< 0.01**
Neuropsychiatric Inventory (NPI) Total	23.9 (± 19.5)	11.1 (± 6.9)	**0.03**
Neuropsychiatric Inventory (NPI) Carer total	17.6 (± 14.4)	10.7 (± 7.9)	0.12
Cornell Scale for Depression in Dementia (CSDD)	6.5 (± 4.3)	3.8 (± 3.0)	0.06
Clinical Dementia Rating (CDR)	1.2 (± 0.5)	0.9 (± 0.2)	0.08

The demographic, clinical and neuropsychological characteristics for the DLB cohort are summarised in *Table 8.1.* Clinical and neuropsychological scores were unavailable for one patient whose condition deteriorated shortly after MIBG scan and precluded further data collection.

The NPI subscale records the frequency and severity of all hallucinations but was used in analysis and discussion as a measure of visual hallucinations, as no subject described hallucinations in any other modalities.

As would be expected, patients with DLB had significantly higher scores in each of three scales measuring core DLB symptoms; UPDRS, NPI Hallucinations subscale and DCFS. A higher total NPI score, indicating greater symptom frequency and/or severity, and a higher BADLS score, indicating greater functional impairment, were also observed in the DLB group when compared to the AD group.

8.2 Late HMR

Our primary MIBG outcome measure, the mean late HMR for DLB cases (1.69, SD ± 0.70) was significantly lower than that of AD cases (2.35, SD ± 0.41; U=57.0, $p<0.01$) (*Figure 8.1*).

Using the HMR of 2.2 to differentiate between "*abnormal*" and "*normal*" HMR values, of 17 cases meeting clinical criteria for probable or possible DLB, 12 had abnormal HMR values, while 5 had normal HMR values. This corresponded to a sensitivity of 71% (95% CI 44-90%) (*Table 8.2*).

Twelve of 16 patients with a clinical diagnosis of AD had a normal MIBG result, corresponding to a specificity of 75% (95% CI 48 - 93%). MIBG result correctly corresponded to clinical diagnosis in 24 of 33 participants, demonstrating an overall accuracy of 73%.

As described in the methods chapter, raters divided MIBG uptake in a myocardial ROI, drawn using a freehand tool, by a reference mediastinal ROI. The mean value of HMRs independently calculated by two practitioners (JK and GR) contributed to this data.

Figure 8.1 Late HMR in DLB and AD patients

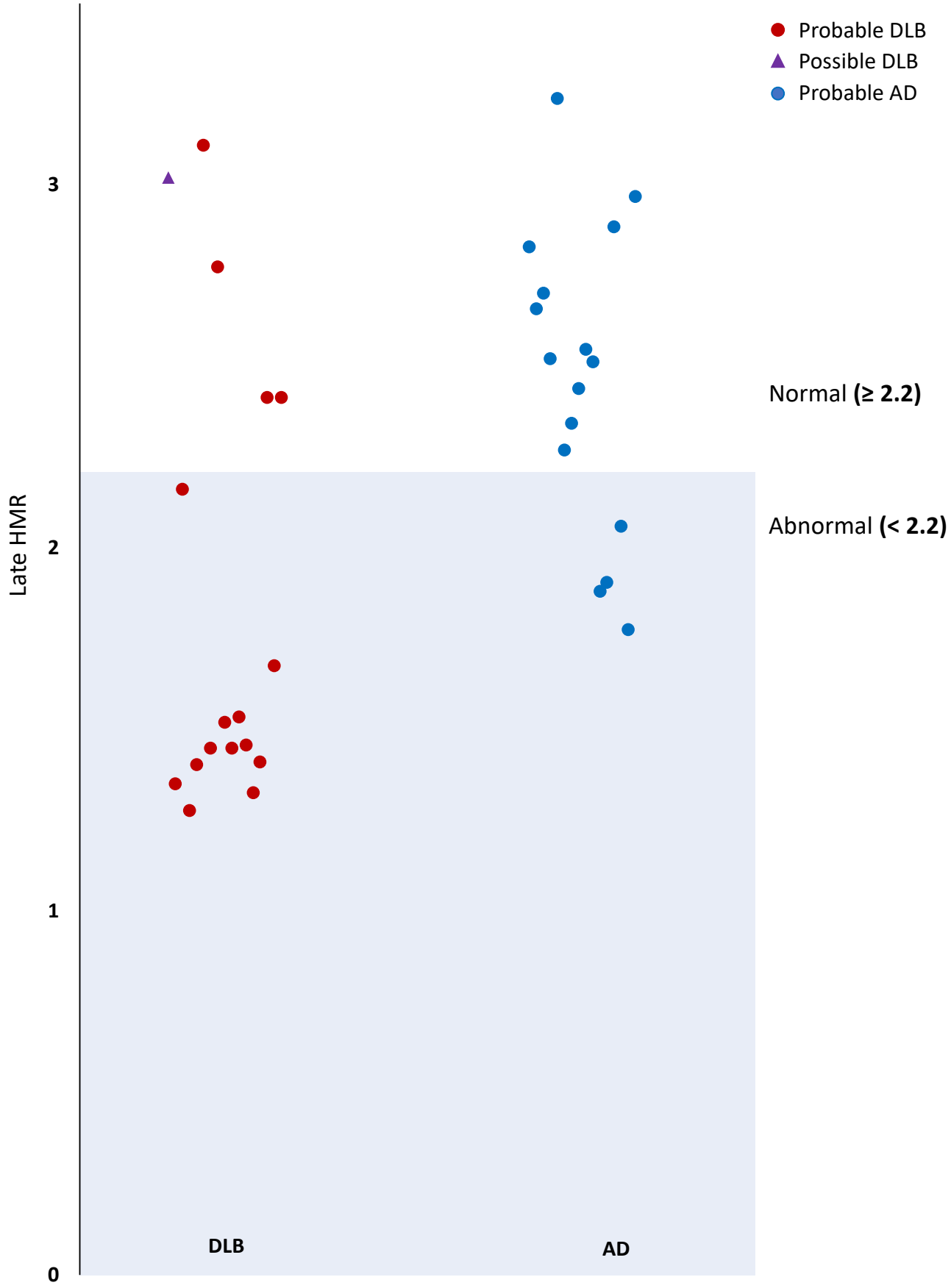


Table 8.2 MIBG result and clinical diagnosis

	Clinical DLB	Clinical AD	Total
MIBG abnormal (HMR < 2.2)	12	4	16
MIBG normal (HMR ≥ 2.2)	5	12	17
Total	**17**	**16**	**33**

The two raters' normal/ abnormal MIBG result agreed on 97% (32/33) of cases (Cohen's kappa = 0.94). In five cases (5/33; 15%) the difference between the two raters' HMRs exceeded 10% of the mean HMR *(Table 8.3)*. In one of these cases (MID027), HMR values reported by each rater would have corresponded to a disagreement over the scans normal/abnormal MIBG status.

Table 8.3 Disagreement between HMR raters

	Clinical Diagnosis	Late HMR (JK)	Late HMR (GR)	Mean HMR	Difference between HMRs/Mean HMR (%)
MID003	Probable AD	2.84	2.46	2.67	14.2%
MID014	Probable DLB	1.21*	1.66*	1.43*	31.5%
MID023	Probable DLB	1.05*	1.34*	1.20*	24.2%
MID027	Probable AD	2.41	2.10*	2.25	13.8%
MID038	Possible DLB	2.60	2.91	2.76	11.2%

**Abnormal MIBG (HMR <2.2)*

8.3 FP-CIT

Of 17 patients with DLB, 14 (82%) had an abnormal FP-CIT result. Two patients (2/16; 13%) with a diagnosis of probable AD also had an abnormal FP-CIT result (*Table 8.4*); both of these cases had a HMR within normal ranges.

Table 8.4 FP-CIT result and clinical diagnosis

	Clinical DLB	Clinical AD	Total
FP-CIT Abnormal	14	2	16
FP-CIT Normal	3	14	17
Total	**17**	**16**	**33**

FP-CIT therefore had a sensitivity of 82% (95% CI 57- 96%) and specificity of 88% (95% CI 62-99%). FP-CIT result correctly corresponded to clinical diagnosis in 28 of 33 participants, demonstrating

an overall accuracy of 85%. None of the cases determined by consensus panel review to have a normal FP-CIT on visual rating had abnormal uptake results on DaTQUANT analysis.

8.4 Agreement between MIBG and FP-CIT

MIBG and FP-CIT concurred in 23 of 33 (70%) of DLB and AD cases (Cohen's kappa = 0.39). FP-CIT findings were abnormal in 11 cases in which MIBG was abnormal (11/16; 69%) and were normal in 12 cases where MIBG findings were normal (12/17; 71%) (*Table 8.5*).

Table 8.5 Consensus between FP-CIT and MIBG findings

	FP-CIT Abnormal	FP-CIT Normal	Total
MIBG Abnormal	11	5	16
MIBG Normal	5	12	17
Total	**16**	**17**	**33**

Five (5/33; 15%) participants with abnormal FP-CIT had a MIBG HMR within normal ranges. Two of these cases (Late HMRs 2.5, 3.0) had a clinical diagnosis of probable AD; the remaining three cases had clinical diagnoses of probable DLB (Late HMRs 2.3, 2.4, 3.2).

Agreement between FP-CIT and MIBG is explored further in receiver operating characteristic (ROC) analysis below.

8.5 Receiver operating characteristic (ROC) analysis

The area under the ROC curve was calculated at 0.79 (95% CI 0.62 – 0.97). The optimum HMR cut-off, as determined by the point of the ROC curve furthest from the diagonal line, is 1.7. This value would be 100% specific and 71% sensitive in differentiating DLB from AD, corresponding to an accuracy of 85% (*Figure 8.2*).

Table 8.6 and *Figure 8.2* demonstrate sensitivity (for detecting DLB), specificity (for AD) and overall diagnostic accuracy at a range of different HMR cut-offs. MIBG sensitivity in this cohort would remain at 71% were any HMR cut-off between 1.7 and 2.3 to be employed; however, a 1.7 cut-off would offer greater specificity (100%) than the 2.2 figure set at the beginning of the study (75%).

Agreement between MIBG and FP-CIT would also have been highest (82%) at a HMR cut-off of 1.7 or 1.5 (*Table 8.6*). Although MIBG would report agreement on the same proportion of abnormal results at cut-offs of both 1.7 and 2.2 (11/16; 69%), the two modalities would agree on a higher proportion of normal results at the 1.7 cut-off (16/17; 94%) than at the 2.2 boundary (12/17; 71%).

Figure 8.2 ROC curve of MIBG sensitivity and specificity in DLB vs AD

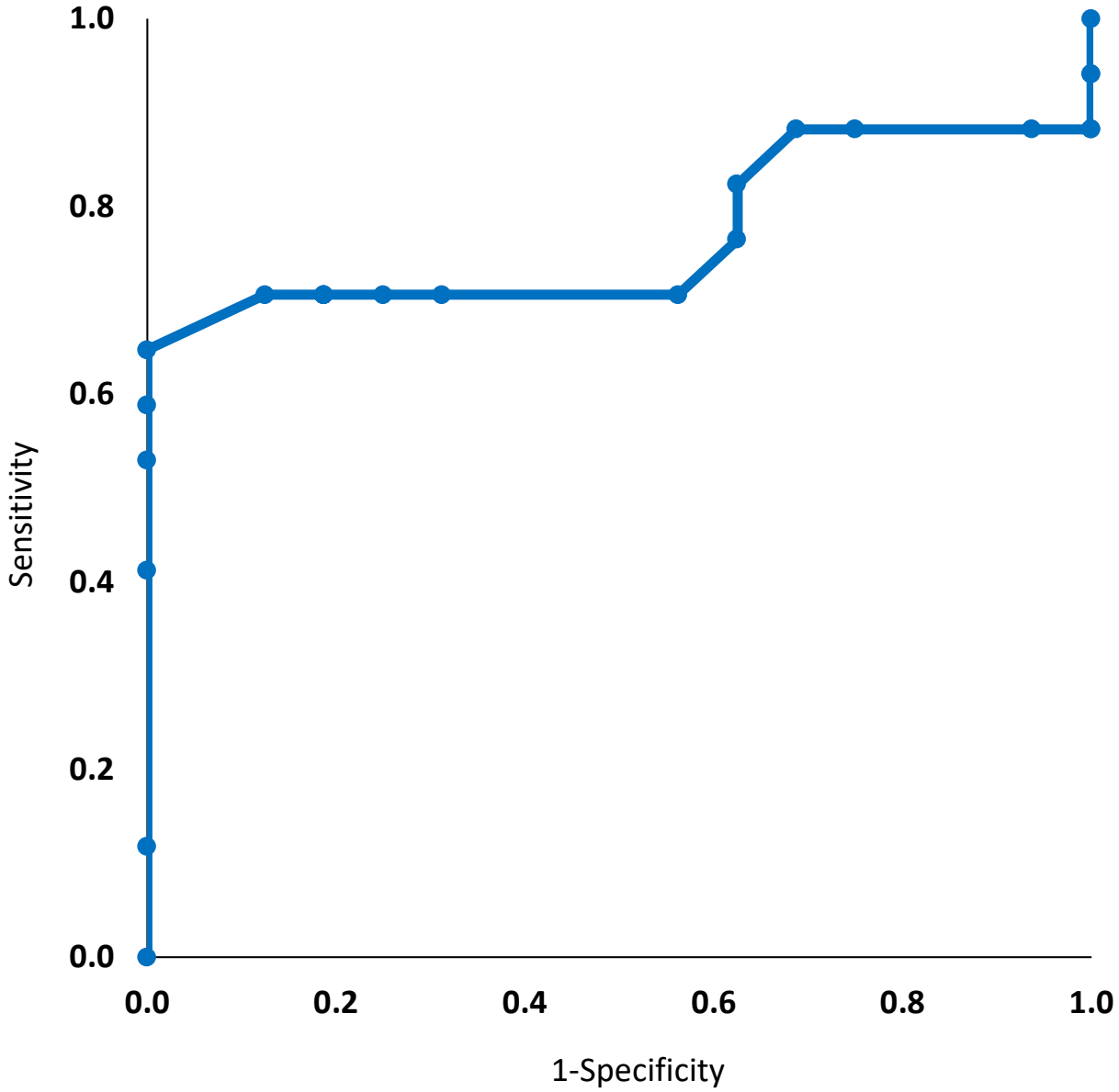


Table 8.6 *MIBG sensitivity, specificity, accuracy and agreement with FP-CIT at a range of HMR cut-off values*

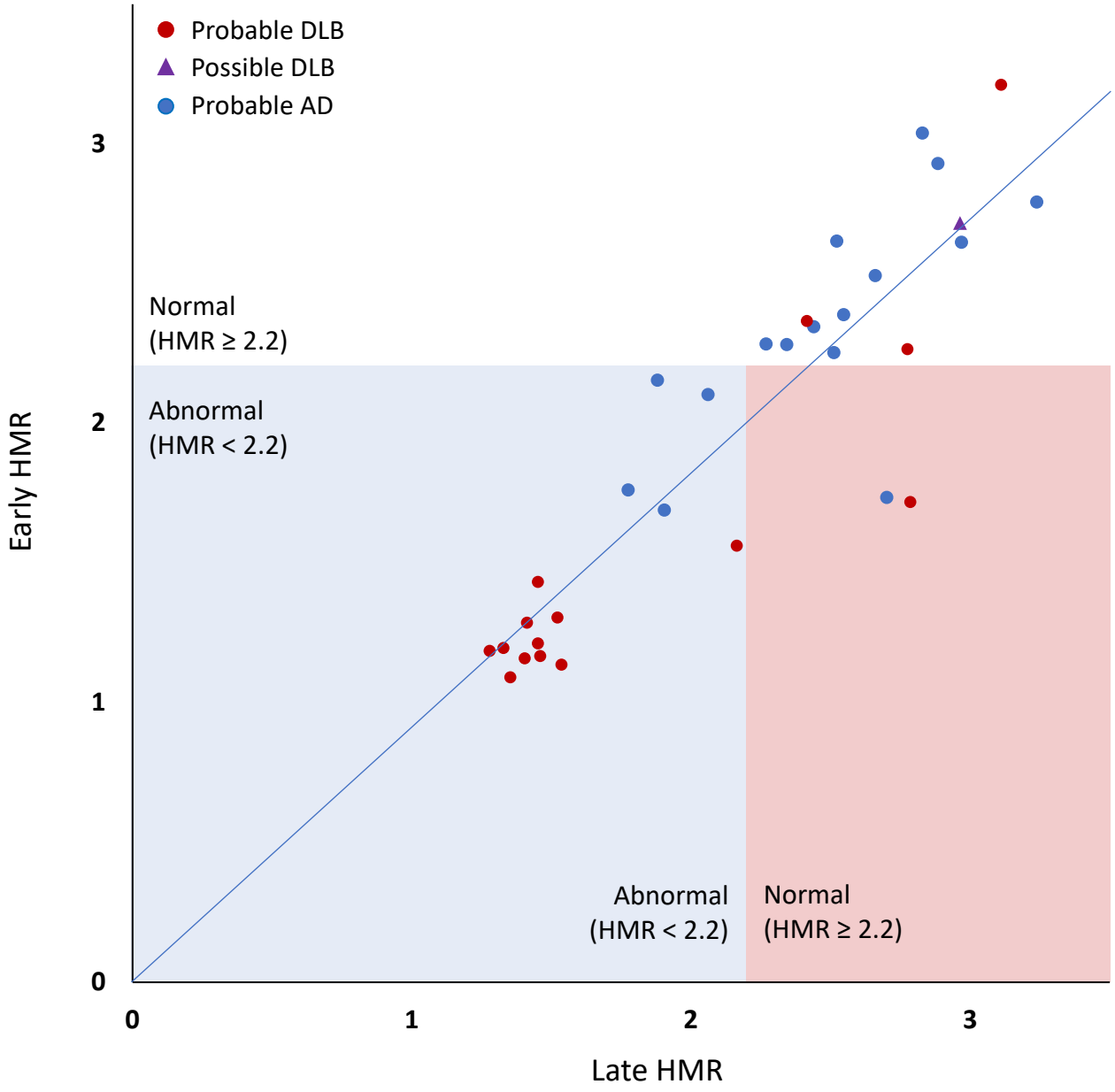
Figures corresponding with the HMR cut-off associated with the highest accuracy (1.7) are displayed in bold.

HMR cut- off	Sensitivity (%)	Specificity (%)	Accuracy (%)	Agreement with FP-CIT		
				Abnormal FP-CIT (%)	Normal FP-CIT (%)	Overall (%)
1.1	0.0	100.0	48.5	0.0	100.0	51.5
1.2	11.8	100.0	54.5	12.5	100.0	57.6
1.3	41.2	100.0	69.7	43.8	100.0	72.7
1.4	52.9	100.0	75.8	56.3	100.0	78.7
1.5	58.8	100.0	78.8	62.5	100.0	81.8
1.6	64.7	100.0	81.8	62.5	94.1	78.7
1.7	**70.6**	**100.0**	**84.8**	**68.8**	**94.1**	**81.8**
1.8	70.6	87.5	78.8	68.8	82.4	75.7
1.9	70.6	81.3	75.8	68.8	76.5	72.7
2.0	70.6	81.3	75.8	68.8	76.5	72.7
2.1	70.6	81.3	75.8	68.8	76.5	72.7
2.2	70.6	75.0	72.7	68.8	70.6	69.7
2.3	70.6	68.8	69.7	68.8	64.7	66.6
2.4	76.5	43.8	60.6	75.0	41.2	57.6
2.5	82.4	37.5	60.6	81.3	35.3	57.6
2.6	82.4	31.3	57.6	87.5	35.3	60.6
2.7	82.4	25.0	54.5	87.5	29.4	57.6
2.8	82.4	18.8	51.5	87.5	23.5	54.5
2.9	94.1	12.5	54.5	87.5	5.8	45.5
3.0	94.1	6.3	.51.5	87.5	0.0	42.4
3.1	94.1	0.0	48.5	93.8	0.0	45.5
3.2	94.1	0.0	48.5	93.8	0.0	45.5
3.3	100.0	0.0	51.5	100.0	0.0	48.4

8.6 Early HMR and Late HMR

We observed a significant correlation between early HMR and late HMR ($r=0.90$, $p<0.01$; $R^2=0.85$) *(Figure 8.3)*. However, two cases, one with a clinical diagnosis of probable DLB, the other a diagnosis of probable AD, demonstrated HMRs within the abnormal range on early imaging, only for their late HMR values to rise into the normal range. These are depicted within the pink area of *Figure 8.3*. One probable DLB case would have demonstrated the same pattern if the HMR 1.7 threshold suggested by ROC analysis had been employed. None of the cases with normal early HMR values had abnormal late HMR values using either *a priori* or *post hoc* cut-offs.

Figure 8.3 *Correlation between early HMR and late HMR with line of best fit*



8.7 Myocardial infarction and late HMR

Five of the 33 patients (15%) recruited to Phase 1 of the study had a history of MI; three with a diagnosis of Probable AD (3/16; 19%), two with a diagnosis of probable DLB (2/17; 12*%) (Table 8.7).* In one of the three AD cases with a history of MI, MIBG yielded a false positive result.

No significant differences were observed between late HMR in patients with a history of MI and those without. No significant difference in age was observed between patients with history of MI and those without (U=49.0, Z=-1.06, r=-0.18, p=0.29*) (Table 8.8).*

Table 8.7 Subjects with a history of MI

	Clinical diagnosis	**FP-CIT result**	**MIBG Late HMR**
MID007	Probable AD	Abnormal	2.5
MID029	Probable AD	Normal	2.9
MID040	Probable AD	Normal	2.1*
MID042	Probable DLB	Abnormal	1.2
MID047	Probable DLB	Normal	2.8**

* *False positive result*
** *False negative result*

Table 8.8 Late HMR in patients with and without a history of MI

	History of MI			**No history of MI**			
	Mean Late HMR (± SD)	Mean Rank	Mean age (± SD)	Mean Late HMR (± SD)	Mean Rank	Mean age (± SD)	**p** (Mean Late HMR)
DLB	1.98 (± 0.67)	10.5	79.0 (± 9.9)	1.64 (± 1.12)	8.8	76.7 (± 8.0)	0.66
AD	2.52 (± 0.42)	10.0	73.3 (± 9.1)	2.31 (± 0.41)	8.2	77.1 (± 6.2)	0.54
Overall	2.30 (± 0.70)	21.2	75.6 (± 8.7)	1.95 (± 0.65)	16.3	76.9 (± 7.1)	0.29

Among these five cases with a history of MI, MIBG demonstrated a sensitivity, specificity and accuracy of 50% (95% CI 1-99%), 100% (95% CI 29-100%) and 80% (95% CI 28-99%), irrespective of whether the *a priori* (2.2) or *post hoc* (1.7) cut-off was used. No alternative cut-off would provide superior accuracy that these values.

8.8 Potentially interacting medications and late HMR

Eighteen of 33 subjects (55%) were prescribed agents identified as capable of influencing MIBG uptake (Giammarile *et al.*, 2008). These comprised six patients with a clinical diagnosis of probable AD (6/16; 38%) and twelve with a diagnosis of probable DLB (12/17; 71%).

No significant differences in late HMR were observed between patients prescribed interacting agents (1.82 ± 0.67) and those not prescribed such medications (2.24 ± 0.59), although this did approach statistical significance (U=92.0, Z=-1.56, r= -0.27, p=0.12). However the fact HMRs were lower in this group is unsurprising as there were proportionately more DLB patients than in the group not prescribed medications *(Table 8.9)*. There was no significant difference in age between the two groups (U=92.5, p=0.12).

Table 8.9 Late HMR in patients prescribed interacting medications

	Prescribed interacting medication			**Not prescribed interacting medication**			
	Mean Late HMR (± SD)	Mean rank	Mean age (± SD)	Mean Late HMR (± SD)	Mean Rank	Mean age (± SD)	**p** (Mean Late HMR)
DLB	1.54 (± 0.58)	8.1	75.4 (± 8.5)	2.01 (± 0.91)	11.2	80.8 (± 4.9)	0.25
AD	2.35 (± 0.52)	9.0	74.7 (± 6.7)	2.35 (± 0.36)	8.2	77.4 (± 6.7)	0.75
Overall	1.82 (± 0.67)	14.6	75.2 (± 7.8)	2.24 (± 0.59)	19.9	78.5 (± 6.2)	0.12

However, it is noteworthy that seven of 18 subjects prescribed potentially interfering medications (39%) were prescribed levodopa compounds; in five of 18 subjects (28%), dopaminergic medication was the only interacting agent prescribed. Every patient prescribed levodopa had a clinical diagnosis of probable DLB and was therefore likely to have a greater degree of cardiac denervation, and a lower HMR, irrespective of medication prescription.

When dopamine was not considered as an interacting medication *(Table 8.10),* no significant difference was identified between late HMR of those prescribed interacting medications (1.96 ± 0.69) and those not prescribed these agents (2.04 ± 0.65; U=128.0, Z=-0.18, r=-0.03, p=0.86). There was no significant difference in age between the two groups (U=92.0, p=0.14).

Table 8.10 Late HMR in patients prescribed interacting medications (excluding dopamine)

	Prescribed interacting medication			Not prescribed interacting medication			
	Mean Late HMR (± SD)	Mean Rank	Mean age (± SD)	Mean Late HMR (± SD)	Mean Rank	Mean age (± SD)	p (Mean Late HMR)
DLB	1.67 (± 0.68)	8.4	75.6 (± 5.3)	1.70 (± 0.75)	9.6	78.2 (± 9.8)	0.63
AD	2.35 (± 0.52)	8.2	74.7 (± 6.7)	2.35 (± 0.36)	9.0	77.4(± 6.7)	0.75
Overall	1.96 (± 0.69)	16.6	75.2 (± 5.7)	2.04 (± 0.65)	17.3	77.8 (± 8.1)	0.86

Four subjects with AD were prescribed amlodipine, a calcium channel blocker; a false positive HMR was observed in two of these four subjects (50%). Three DLB patients were prescribed two or more agents. Three false negative results were observed among this group (3/12; 25%), comprising patients prescribed diltiazem and venlafaxine *(Table 8.11).*

Table 8.11 Subjects prescribed medications potentially interacting with MIBG uptake

	Clinical diagnosis	FP-CIT result	MIBG Late HMR	Agents prescribed
MID025	Probable AD	Normal	2.8	Mirtazapine^{+}
MID035	Probable AD	Normal	2.4	Diltiazem^{++}
MID039	Probable AD	Normal	1.7*	Amlodipine^{+}
MID040	Probable AD	Normal	2.9	Amlodipine^{+}
MID044	Probable AD	Normal	1.8*	Amlodipine^{+}
MID047	Probable AD	Abnormal	2.5	Amlodipine^{+}
MID002	Probable DLB	Abnormal	1.1	Quetiapine^{+}
MID004	Probable DLB	Abnormal	1.6	Dopamine^{+}
MID007	Probable DLB	Abnormal	1.2	Dopamine^{+}
MID015	Probable DLB	Abnormal	2.3**	Amlodipine^{+}, dopamine^{+}
MID016	Probable DLB	Abnormal	1.3	Dopamine^{+}
MID018	Probable DLB	Abnormal	1.2	Amlodipine^{+}
MID019	Probable DLB	Abnormal	1.1	Dopamine^{+}, mirtazapine^{+}, quetiapine^{+}
MID020	Probable DLB	Abnormal	1.2	Dopamine^{+}
MID023	Probable DLB	Abnormal	1.2	Dopamine^{+}
MID028	Probable DLB	Abnormal	1.3	Amlodipine^{+}, salbutamol^{+}, salmeterol^{+}
MID029	Probable DLB	Normal	2.8**	Diltiazem^{++}
MID031	Probable DLB	Abnormal	2.4**	Venlafaxine^{+}

* *False positive result* + *Theoretical interaction*
** *False negative result* ++ *Probable interaction*

The sensitivity, specificity and accuracy of MIBG for patients prescribed potentially interfering medications (including dopamine) were 75% (95% CI 43-95%), 67% (95% CI 22-96%) and 72% (95% CI 47-90%) using the *a priori* (2.2) cut off and 75% (95% CI 43-95%), 83% (95% CI 35-100%) and 78% (95% CI 52-94%) using the *post hoc* (1.7) cut-off. When patients prescribed dopamine were excluded, the sensitivity, specificity and accuracy of MIBG were 43% (95% CI 10-82%), 67% (95% CI 22-96%) and 54% (95% CI 25-81%) using the *a priori* cut-off and 43% (95% CI 10-82%), 83% (95% CI 36-100%) and 62% (95% CI 32-86%) using the *post hoc* cut off.

The optimum cut-off in patients prescribed interfering medication was 1.6, corresponding to a sensitivity, specificity and accuracy of 43% (95% CI 10-82%), 100% (95% CI 54-100%) and 69% (95% CI 39-91%). The same value was the optimum cut-off when patients prescribed dopamine were excluded; MIBG would demonstrate a sensitivity, specificity and accuracy of 43% (95% CI 10-82%), 100% (95% CI 54-100%) and 69% (95% CI 39-91%) were it to be used.

8.9 Late HMR and core DLB symptoms

Late HMR showed a significant negative correlation with the hallucinations subscale of NPI in DLB patients (r=-0.56, p=0.03) *(Table 8.12),* indicating the more abnormal the cardiac imaging, the more severe and frequent were hallucinations. Late HMR did not correlate significantly with any other cognitive, functional or core symptom scale.

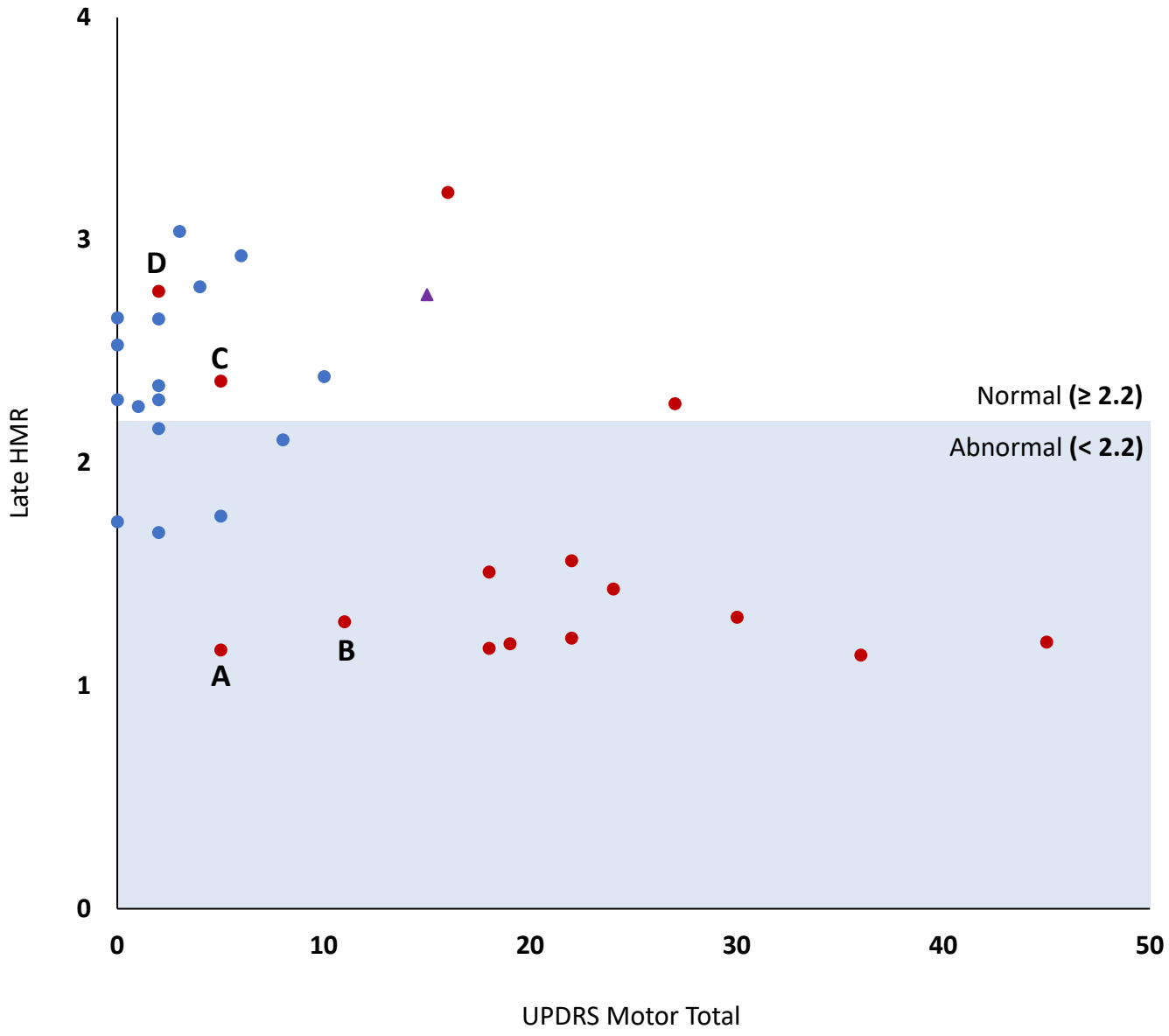
Table 8.12 Correlation between late HMR and cognitive, functional and core DLB symptom measures

	DLB (n=17)	
	Pearson r	**p**
Revised Addenbrooke's Cognitive Examination (ACE-R)	0.28	0.27
Mini-Mental State Examination (MMSE)	0.30	0.29
Bristol Activities of Daily Living Scale	-0.33	0.19
Instrumental Activities of Daily Living Scale	0.36	0.23
Unified Parkinson's Disease Rating Scale (UPDRS) Motor subscale	-0.38	0.14
Neuropsychiatric Inventory (NPI) Hallucinations subscale	-0.56	**0.03**
Dementia Cognitive Fluctuations Scale (DCFS)	-0.43	0.11

FP-CIT's possible suboptimal sensitivity in patients without parkinsonism (O'Brien *et al.*, 2014b) necessitates exploration of the relationship between late HMR and UPDRS motor subscale score; MIBG would be of greatest clinical benefit in patients for whom FP-CIT would be likely to produce a false negative result.

Four (4/17; 24%) of DLB cases, all meeting criteria for probable DLB, scored less than 15 on the UPDRS motor subscale, with three (3/17; 18%) scoring eight or less (*Figure 8.4*). Two cases (A and B), had both an abnormal FP-CIT and abnormal MIBG result; one case (C) had an abnormal FP-CIT and normal (HMR=2.4) MIBG result; and one case (D) had a normal FP-CIT, and normal (HMR =2.8) MIBG. Thus, the only DLB cases without parkinsonism in which MIBG produced an abnormal result were those who also had abnormal FP-CIT findings. The status of each of these four cases were the same irrespective of the use of the predetermined HMR cut-off of 2.2 or the 1.7 cut-off determined by sensitivity analysis.

Figure 8.4 Correlation between late HMR and UPDRS motor subscale score



8.10 DLB patients with normal FP-CIT

In addition to the 33 patients recruited to the study, we identified six participants meeting diagnostic criteria for probable or possible DLB but who recently had a normal FP-CIT scan. These patients had significantly lower levels of functional impairment as measured by the Bristol Activities of Daily Living Scale (t(19)= 2.5,p=0.02), and lower levels of parkinsonism (t(20)=2.3 , p=0.03) than participants recruited to the main study cohort *(Table 8.13).*

Table 8.13 Demographic and clinical characteristics of DLB patients in the main study cohort and FP-CIT negative cohort

	DLB Main cohort (n=17) Mean score (± SD)	**DLB Normal FP-CIT cohort (n=6)** Mean score (± SD)	**p**
Age in years at MIBG (± SD)	77.0 (± 7.9)	78.0 (± 4.4)	0.77
Gender male/female (% Male)	15/2 (88%)	5/1 (83%)	0.76
Revised Addenbrooke's Cognitive Examination (ACE-R)	66.6 (± 15.1)	69.2 (± 7.6)	0.58
ACE-R subscale Attention and Orientation	13.9 (± 3.6)	14.7 (± 2.5)	0.69
ACE-R subscale Memory	13.4 (± 5.1)	13.2 (± 4.4)	0.63
ACE-R subscale Fluency	6.4 (± 3.3)	6.7 (± 1.5)	0.91
ACE-R subscale Language	22.3 (± 5.1)	22.8 (± 2.6)	0.87
ACE-R subscale Visuospatial	10.6 (± 4.1)	11.8 (± 2.3)	0.80
Mini-Mental State Examination (MMSE)	22.1 (± 4.9)	23.3 (± 3.1)	0.49
Bristol Activities of Daily Living Scale	22.3 (± 13.2)	12.3 (± 5.0)	**0.02**
Instrumental Activities of Daily Living Scale	2.6 (± 1.9)	4.3 (± 1.0)	0.05
Unified Parkinson's Disease Rating Scale (Motor subscale)	32.8 (± 23.4)	7.8 (± 7.7)	**0.03**
Neuropsychiatric Inventory (Hallucinations subscale)	2.1 (± 2.5)	1.0 (± 2.5)	0.35
Dementia Cognitive Fluctuations Scale	10.9 (± 3.7)	9.0 (± 3.5)	0.30
Mayo Sleep Questionnaire	6.8 (± 2.3)	9.3 (± 0.6)	0.09
Neuropsychiatric Inventory (Total)	23.9 (± 19.5)	17.7 (± 13.6)	0.48
Neuropsychiatric Inventory (Carer total)	17.6 (± 14.4)	15.0 (± 9.9)	0.69
Cornell Scale for Depression in Dementia	6.5 (± 4.3)	5.7 (± 2.7)	0.68
Clinical Dementia Rating (CDR)	1.2 (± 0.5)	0.7 (± 0.3)	0.06

All FP-CIT data collected from these recent scans underwent reprocessing and review by a blinded panel using a methodology identical to that employed elsewhere in the study (*Chapter 7.3.8*). In two of six cases (33%), the FP-CIT result determined by our blinded study panel contradicted the normal result previously reported by local nuclear medicine practitioners *(Table 8.14).*

Two of six recruited cases showed late HMR results <2.2, both with a diagnosis of probable DLB. One of these cases (MID005) had a history of MI and calcium channel blocker prescription and comprised one of the cases in whom FP-CIT results was revised to abnormal. The second case with an abnormal late HMR (MID009) was subject to neither significant cardiac history nor prescription of interfering medications.

Table 8.14 DLB patients with normal FP-CIT

Study ID	Sex	Age	Clinical diagnosis	Review panel FP-CIT result	Late HMR	History of MI	Interfering medications
MID005	M	76	Probable DLB	Abnormal	**1.7**	Yes	Diltiazem^{++}
MID009	M	78	Probable DLB	Normal	**1.4**	Yes	None
MID022	M	71	Probable DLB	Normal	3.3	No	None
MID032	M	81	Probable DLB	Normal	3.3	No	None
MID013	F	78	Possible DLB	Normal	2.7	No	None
MID017	M	84	Possible DLB	Abnormal	2.7	No	None

8.11 Summary

Our study recruited 33 patients; 17 DLB and 16 AD. The mean HMR for patients with clinical DLB was significantly lower than that of cases with AD. Using the *a priori* cut-off set for the study (2.2), based on the Japanese literature (Yoshita *et al.*, 2015), MIBG demonstrated a sensitivity of 71% and specificity of 75% for separating DLB from AD, compared with 82% sensitivity and 88% specificity for FP-CIT. MIBG and FP-CIT results concurred in 70% of cases recruited to the study. ROC analysis identified an optimum HMR cut-off of 1.7, which would be 71% sensitive and 100% specific in differentiating DLB from AD.

Eighteen of 33 subjects (55%) participating in the study were prescribed agents identified as potentially influencing MIBG uptake, and five subjects (15%) recruited to the study had a history of MI. No significant differences were identified between patients exposed to these factors and those who were not, but calcium channel blockers were prescribed in participants with both false positive and false negative results.

With the exception of the negative correlation between late HMR and the hallucinations subscale of the NPI, no significant relationships were identified between late HMR and cognitive, clinical or functioning scales in DLB patients. MIBG only reported abnormal values for DLB patients without parkinsonism where FP-CIT had also reported abnormal findings.

Six additional patients were recruited to the study, who each met criteria for probable or possible DLB but who had recently had a normal FP-CIT scan in clinical services. In two of these cases, independent FP-CIT review reported an abnormal study contrary to that reported by local nuclear medicine practitioners. Two cases with a diagnosis of probable DLB had abnormal MIBG findings, one of whom had neither a history of MI, interfering medication, nor revision of FP-CIT result by independent panel.

Chapter 9

MIBG cardiac scintigraphy as a biomarker for DLB - discussion

9.1 Introduction

Our discussion will, in keeping with the theme of this thesis, discuss the results of this study from a clinical perspective. We will first discuss the sensitivity and specificity of MIBG in this cohort using the *a priori* cut-off derived from phantom calibration (Nakajima *et al.*, 2012) and a previous multicentre study (Yoshita *et al.*, 2015). This will be followed by exploration of another of the aims of the study; determination of an appropriate HMR cut-off for a UK population. In doing so we will examine the results of ROC analysis and the factors contributing to the differences observed between our *a priori* and *post hoc* optimum cut-offs. The relationship between interacting medications, MI and late HMR, will then be considered before discussion of MIBG utility in DLB patients without parkinsonian features and in those who had previously had normal FP-CIT scans. Finally, we will discuss how our findings contribute to our understanding of DLB pathogenesis.

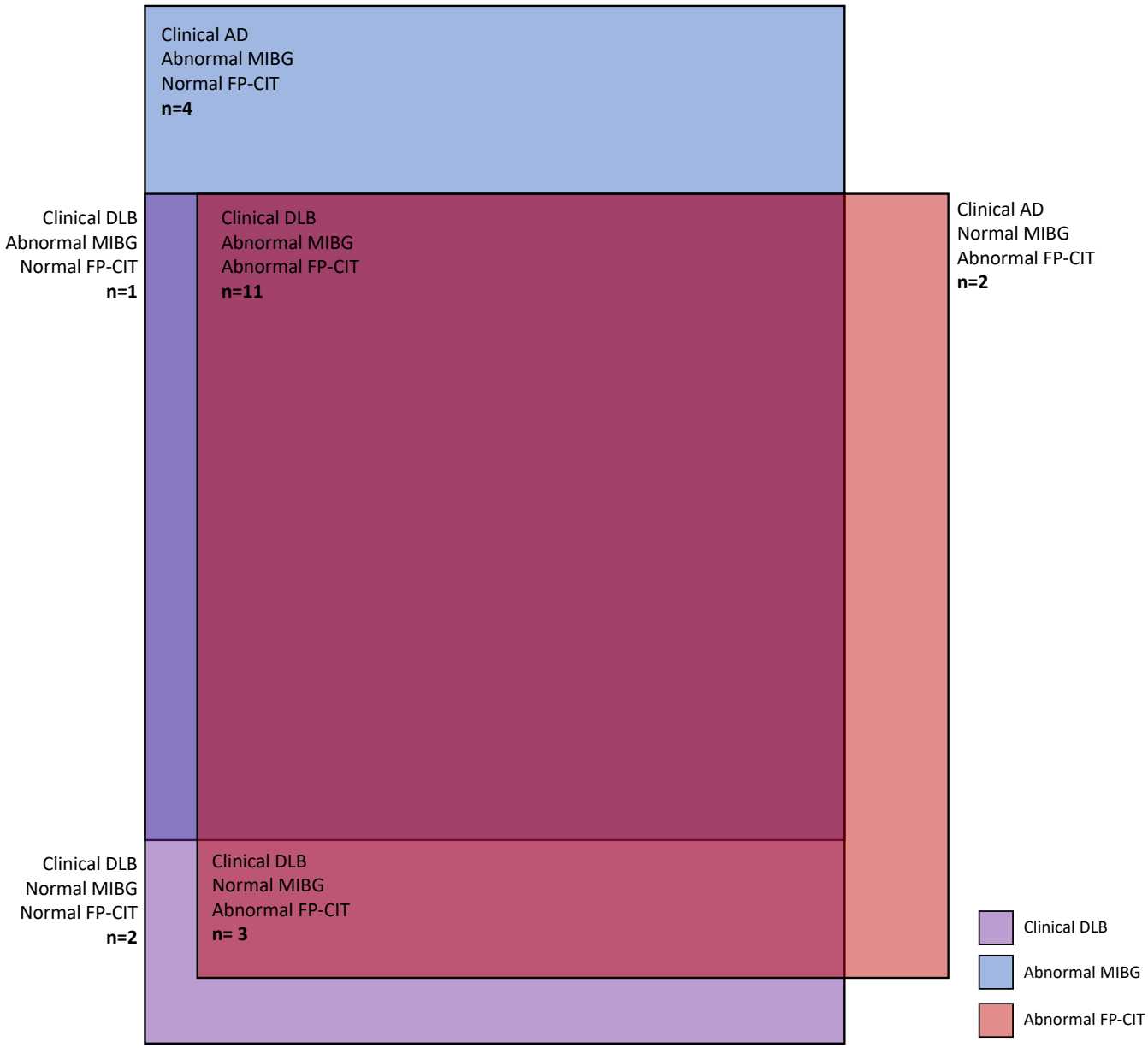
9.2 Sensitivity

Our study design adopted a necessarily exploratory approach to HMR cut-off in a population in whom MIBG had not previously been researched. However, for our initial analysis we adopted the mean HMR values from the largest MIBG DLB sample published to date (Yoshita *et al.*, 2015) and a phantom calibration process used to standardise multiple machines contributing to the same study (Nakajima *et al.*, 2012). This process identified an early and late HMR threshold of 2.2 to distinguish between normal (≥2.2) and abnormal (<2.2) studies. Although we had anticipated that our data would identify an alternative *post hoc* HMR cut-off for our cohort, it is important to first discuss the utility of MIBG using the *a priori* values adopted.

We found that MIBG demonstrated a sensitivity of 71% in our study cohort. This is considerably lower than the 93% reported by meta-analysis (Treglia and Cason, 2012), comprising mostly Japanese studies, but is also lower than that reported by Italian (93%) and Spanish (94%) groups (Estorch *et al.*, 2008; Tiraboschi *et al.*, 2016). This sensitivity is, however, very similar to the 69% reported by a multicentre Japanese trial (Yoshita *et al.*, 2015), which assessed the largest single cohort in the field to date.

When MIBG results were compared directly with FP-CIT results the two investigations concurred in 70% of cases. Every combination of clinical diagnosis, MIBG result and FP-CIT result, with the exception of AD cases testing positive for both MIBG and FP-CIT (n=10), were represented in this sample (*Figure 9.1*).

Figure 9.1 *Relationships between clinical diagnosis, MIBG result and FP-CIT result in the main cohort*



Five of 17 patients meeting diagnostic criteria for possible or probable DLB (29%) had a late HMR within the normal range. Three of these five cases (60%) had an abnormal FP-CIT scan *(Table 9.1).*

Possible reasons for the false negative status of these patients, and the effect of these on overall sensitivity, will be explored in turn; the inclusion of participants with possible DLB, the accuracy of clinical diagnosis, the effect of medications and MI, and the HMR cut-off used by the study.

Table 9.1 DLB patients with normal late HMR values

	Clinical diagnosis	FP-CIT result	Late HMR	History of MI	Interacting medications
MID010	Probable DLB	Abnormal	3.2	no	none
MID015	Probable DLB	Abnormal	2.3	no	Amlodipine, dopamine
MID029	Probable DLB	Normal	2.8	yes	Diltiazem
MID031	Probable DLB	Abnormal	2.4	no	Venlafaxine
MID038	Possible DLB	Normal	2.8	no	none

We adopted clinical diagnosis, as determined by a panel of three independent practitioners, as our gold standard measurement. This was on the basis of both the validity of this process as an alternative to *post-mortem* assessment (McKeith *et al.*, 2000b), and the desire for our study to maintain a clear clinical relevance. However, the 85% specificity associated with this use of third consensus criteria (McKeith *et al.*, 2000b) compels us to consider the possibility that a proportion of patients recruited to our DLB group may have alternative diagnoses not associated with cardiac denervation. This suspicion should be greatest in two cases with both normal FP-CIT and normal MIBG (*Table 9.1*; MID029, MID038). The reported sensitivity of FP-CIT of 80% in a neuropathological cohort (Thomas *et al.*, 2017a) requires consideration that either or both of these cases could represent other dementia subtypes misdiagnosed by the clinician panel.

Conversely, the abnormal FP-CIT results for the remaining three DLB cases with normal MIBG (MID010, MID015, MID031), make misdiagnosis in this group unlikely, particularly given the combined sensitivities of FP-CIT and independent clinician rating. However, pathologies such as FTD, vascular parkinsonism, MSA and PSP could all produce false positive FP-CIT results (Zijlmans *et al.*, 2007; Vlaar *et al.*, 2008; Morgan *et al.*, 2012) and can exhibit clinical features suggestive of DLB, without compromising cardiac innervation (Kashihara and Yamamoto, 2006; Novellino *et al.*, 2010; Navarro-Otano *et al.*, 2014). Attention was paid in each case to exclude these conditions during the assessment process, by examination of structural imaging and detailed neurological examination of each patient (JK), but the possibility of such cases influencing our results cannot be excluded.

It is also entirely possible that these five cases, and in particular, the three cases with abnormal FP-CIT, could have Lewy body pathology without autonomic cardiac involvement; Orimo *et al*. (2016) reported normal MIBG results in two of thirteen (15%) neuropathologically confirmed DLB cases, while Beach *et al.* (2010) detected α-synuclein in the epicardium and ventricles of one of four (25%) DLB subjects. Thus our 71% sensitivity could be an accurate measure of the proportion of DLB patients with cardiac sympathetic α-synuclein pathology.

Although unlikely, the possibility that our HMR threshold of 2.2 was too low to detect denervation in these five false negative cases should be explored. A higher cut-off of 2.9, for example, would have increased sensitivity to 94%. There are a number of arguments which strongly suggest that a higher HMR threshold would not be justifiable for our study population. The first is that our HMR cut-off of 2.2 already exceeds all cut-off points in published literature; with the exception of Yoshita *et al*'s 2.1 *post hoc* cut-off, HMR thresholds, where reported, have ranged between 1.4 and 1.8 (Yoshita *et al.*, 2006, 2015; Wada-Isoe *et al.*, 2007; Estorch *et al.*, 2008; Novellino *et al.*, 2010; Tiraboschi *et al.*, 2016).

Secondly, any increase in HMR threshold would have affected the specificity of MIBG in identifying AD cases. In the specialist setting in particular, the high specificity of a test is arguably more important than a high sensitivity and compromising the former to enhance the latter would diminish its clinical value. The late HMR of three of five false negative DLB cases far exceeded the 2.2 cut-off, the highest of which (MID010) was the single highest HMR value recorded in the entire cohort. Increasing the cut-off to 2.8, although increasing sensitivity, would have lowered specificity to only 13%.

Finally, the optimum HMR cut-off determined by ROC analysis suggested a lower HMR threshold (1.7), rather than a higher one. Thus, it is unlikely that our reported sensitivity can be attributed to a low HMR cut-off. HMR cut-off is discussed further in the following section.

Medications or past medical history of MI were both unlikely to have produced false negatives in this study. Although some calcium channel-blocking medications have been implicated in elevating late HMR (Jacobson and Travin, 2015), any agents prescribed to patients enrolled in our study were more likely to decrease than increase MIBG uptake. These are discussed further in *9.6.* Similarly, a history of MI would be more likely to adversely influence specificity than sensitivity.

A sensitivity of 71% is not a strongly persuasive argument for the routine clinical use of MIBG, but the variation in DLB detection rates, as discussed in previous chapters, makes any tool capable of minimising false negative cases in the DLB diagnostic process useful.

9.3 Specificity

Our reported specificity for distinguishing AD from DLB was 75%. This was lower than the that reported by Japanese and European groups, who reported specificity ranging from 89% to 100% (Estorch *et al.*, 2008; Treglia and Cason, 2012; Yoshita *et al.*, 2015; Tiraboschi *et al.*, 2016).

Four of twelve cases (33%) meeting diagnostic criteria for probable AD demonstrated abnormal late HMR values (*Table 9.2*). We will adopt the same approach in discussing factors contributing to false positive cases, and thus specificity, as we did for false negative cases and sensitivity.

Table 9.2 Probable AD cases with abnormal late HMR

Study ID	Clinical diagnosis	FP-CIT result	Late HMR	History of MI	Interacting medications
MID001	Probable AD	Normal	1.7	no	none
MID039	Probable AD	Normal	1.7	no	none
MID042	Probable AD	Normal	2.1	yes	none
MID044	Probable AD	Normal	1.8	no	none

None of the cases with probable AD recruited to the study were prescribed medications identified as capable of inhibiting MIBG uptake. Only one case (MID042) had a history of MI, although it is noteworthy that this case's late HMR was the highest of the four false positive cases.

The same limitations of both the independent clinician panel and FP-CIT imaging discussed previously apply to the false positive cases. The combined high sensitivity of third consensus criteria (83%) and FP-CIT (80%) (McKeith *et al.*, 2000b, 2007) suggest that there are unlikely to be DLB cases among those listed in *Table 9.2*. Furthermore, the high specificity of NIA-AA criteria (95%) (Harris *et al.*, 2015) make inclusion of other subtypes such as FTD, doubtful, and such subtypes would be unlikely to demonstrate impaired MIBG uptake.

Consideration of the late HMR cut-off is again important when considering the role of specificity. All four of the late HMRs of false positive cases listed above fell between 1.7 and 2.1. Were we to

use a threshold of 1.7, a figure similar to that employed in several previous studies (Yoshita *et al.*, 2006; Wada-Isoe *et al.*, 2007; Tiraboschi *et al.*, 2016) and the optimum cut-off suggested by ROC analysis in this cohort, this would increase the specificity of MIBG in this group to 100%. Doing so would have no effect on the sensitivity, which would remain at 71%.

Taken together, the sensitivity (71%) and specificity (75%) associated with our *a priori* HMR cut-off suggest that MIBG could represent a useful diagnostic test but is not strongly persuasive in favour of routine clinical use. This would appear to concur with the findings of multicentre studies investigating the utility of FP-CIT (sensitivity 78%, specificity 90%) and MIBG (sensitivity 69%, specificity 89%) (McKeith *et al.*, 2007; Yoshita *et al.*, 2015)

However, the 71% sensitivity and 100% specificity offered by the 1.7 cut-off is encouraging, particularly given the comparatively inferior specificity for AD of FP-CIT (McKeith *et al.*, 2007), and merits further discussion of HMR cut-off. We did also observe abnormal MIBG findings in one of the three DLB subjects in our main cohort with normal FP-CIT. While this represents a small sample, it could suggest that MIBG may have a second-line diagnostic role in FP-CIT negative patients.

MIBG may yet prove useful in subgroups of DLB patients beyond the scope of this study. These may include prodromal DLB subjects, challenging diagnostic cases, or those with specific structural imaging biomarkers. Larger studies, adopting a similar inclusive approach towards recruitment as ours, could help further understanding of the role that MIBG should play in routine clinical practice.

9.4 Practical considerations in MIBG use

Although our findings regarding utility may support FP-CIT in preference to MIBG, other factors may encourage the use of the latter in clinical DLB diagnosis. Firstly, MIBG could be a more tolerable investigation than FP-CIT among cognitively impaired or frail patients, necessitating less time for data acquisition and less overall time spent by the patient and carer in the hospital. Planar MIBG images are acquired over ten minutes, with the patient in a seated or semi-recumbent position, compared to FP-CIT, which requires the patient to lie supine and still for a 20-minute period. Early planar MIBG images are collected 20 minutes following radioligand injection, while FP-CIT data acquisition takes place three to six hours after injection. We did not, as part of

this study examine the clinical utility of early HMR but did observe a significant correlation between early and late MIBG uptake, and in only two of 33 cases (6%) did the normal/ abnormal status differ between early and late scans. Yoshita *et al*. (2015) noted the comparable utility of data from the two acquisition periods, and the practical and economic potential of shorter investigations encourage further research into the use of early HMR.

Secondly, MIBG confers a lower dose of radiation than FP-CIT when used in the quantities employed in our study (MIBG 1.5mSv, FP-CIT 4.35mSv) and importantly, MIBG (£220 per scan) is cheaper than FP-CIT (£630 per scan). FP-CIT is currently better established in UK services and is supported by a more mature evidence base in comparison to that of MIBG (Sonni *et al.*, 2017), but these practical considerations, particularly around tolerability, could support the use of MIBG in selected patients, such as those unable to tolerate longer scans.

9.5 Late HMR cut-off

We have discussed how the observed sensitivity and specificity of MIBG are greatly affected by the level of the threshold between a normal and abnormal scan; the late HMR cut-off. Determination of an accurate cut-off HMR for use in a UK population was a key aim of the study.

Below we will discuss the factors that could contribute to the differences between our *a priori* cut-off, derived from a previous multicentre study (Yoshita *et al.*, 2015), and our *post hoc* cut-off. These causes can be considered as related to acquisition parameters or patient-based factors.

As discussed in the methods section, our *a priori* threshold of 2.2 was determined by the phantom calibration method used to standardise practice across ten sites contributing to a Japanese multicentre MIBG study (Nakajima *et al.*, 2012; Yoshita *et al.*, 2015). This helped to account for some differences between our methodology and that of other studies; notably our use of medium energy general purpose collimators (MEGPs), rather than the low energy high resolution (LEHR) collimators used in other studies. MEGPs maximise contrast between areas of specific uptake and non-specific uptake, important in determining the boundaries of cardiac ROIs. The thicker septa of MEGPs minimise penetration from the high energy photons contributing to background signal, thus producing higher HMRs (Chen, Cao and Dilsizian, 2011; Inoue *et al.*, 2014). Despite the calibration process, however, we found that the optimum *post hoc* cut-off was lower than the *a*

priori cut-off derived and converted from previous studies. It is therefore unlikely that collimator choice contributed to the difference between our two cut-offs.

This calibration process, while helping to address some differences in parameters between Japanese centres and our practice did not consider a number of patient-specific factors affecting MIBG uptake and quantification. These factors could have contributed to the variation in accuracy, and optimum cut-off observed between this study and others.

Although the method for HMR quantification through comparison of ROIs is a constant throughout MIBG research, accurate analysis using this method is not without its issues. Among these is the effect of non-cardiac MIBG uptake. Scattered radiation from liver and lungs can adversely influence the accurate definition of manually drawn cardiac ROIs. Unintended inclusion of such uptake in the cardiac ROI can therefore raise the HMR and produce a false negative result (*Figure 9.2*) (Verberne *et al.*, 2009; Verschure *et al.*, 2015).

As this is dependent on the size and position of the left lobe of the liver (Verberne *et al.*, 2009), this can vary greatly between patients. MIBG SPECT has been shown to demonstrate a superior sensitivity to planar MIBG by reducing the number of the false negative scans caused by non-cardiac uptake in a cohort of PD patients (Oh *et al.*, 2015). However, while non-cardiac MIBG uptake could influence measurement of HMR on an individual patient basis, it is unlikely this would systematically affect either one of our AD or DLB groups more than the other, and thus unlikely to account for the large difference between *a priori* and *post hoc* cut-offs.

Figure 9.2 High MIBG uptake in liver and lungs of a study participant

The uptake in the liver and lungs makes visual discernment of the cardiac border challenging. The cardiac ROI may therefore inadvertently include uptake in other tissues, thus raising the HMR.

In addition to these image acquisition factors, a number of factors related to our patient cohort could have contributed towards the difference between our *a priori* and *post hoc* cut-offs. The 1.7 cut-off suggested by ROC analysis, although more comparable to the cut-offs cited by previous MIBG studies than the 2.2 *a priori* margin used *(Table 9.3)*, effectively represents a lower threshold of MIBG uptake in our study cohort when the fact that MEGPs were used is considered.

One such patient factor is obesity which, due to attenuation and scatter, has been shown to significantly decrease recorded cardiac MIBG counts and increase non-specific background counts (Pellegrino *et al.*, 2015). This is worth particular consideration given that 26.9% of the UK adult population is obese, compared to 4.4% of the Japanese population (Organisation for Economic Co-operation and Development, 2017). Thirty four percent of adults in North-East England, from where our participants were recruited, meet criteria for obesity (NHS Digital, 2017). Although we did not measure body mass index (BMI) in our participants, the relative preponderance of obesity

in the general population might have been reflected in our study population and it is possible that this could have contributed towards this lower HMR.

Table 9.3 Collimator type, HMR cut-off, sensitivity and specificity of previous MIBG studies to date

Study		**Collimator**	**Late HMR cut-off**	**Sensitivity** (95% CI)	**Specificity** (95% CI)
Watanabe *et al.* (2001)		NR	1.6*	100% (72-100)	100% (69-100)
Yoshita *et al.* (2001)		LEHR	1.6*	100% (91-100)	100% (92-100)
Oide *et al.* (2003)		LEHR	1.9*	100% (69-100)	100% (69-100)
Hanyu *et al.* (2006)		LEHR	1.8*	100% (82-100)	92% (79-98)
Yoshita *et al.* (2006)		LEHR	1.7	100% (91-100)	100% (92-100)
Estorch *et al.* (2008)		LEHR	1.4	94% (74-100)	96% (80-100)
Wada-Isoe *et al.* (2008)		LEHR	1.8	100% (83-100)	91% (75-98)
Noguchi-Shinohara *et al.* (2009)		LEHR	NR	100% (77-100)	100% (77-100)
Novellino *et al.* (2010)		NR	1.4*	100% (66-100)	100% (54-100)
Treglia *et al.* (2012)		NR	1.6	90% (67-98)	91% (57-100)
Yoshita *et al.* (2015)		LEHR, MEGP	2.1	69% (57-81)	89% (80-98)
Tiraboschi (2016)		NR	1.6	93% (78-99)	100% (88-100)
Thesis	*a priori*	MEGP	2.2	71% (44-90)	75% (48-93)
	post hoc	MEGP	1.7	65% (38-86%)	100% (79-100)

LEHR *Low energy high resolution*

MEGP *Medium energy general purpose*

NR *Not reported*

*** *Estimated*

Although we found no association between history of MI and HMR (HMR was higher in DLB patients with MI), ischaemic heart disease, even in the absence of previous MI, could have contributed towards subtle changes in cardiac denervation as sympathetic innervation is more susceptible to ischaemia than cardiac myocytes (Dae *et al.*, 1995). This is particularly important when we consider that males, among whom MI is three times more common (Bhatnagar *et al.*, 2015), comprised a higher proportion of DLB cases (15/17; 88%) in our study than the 38-70% seen in preceding MIBG studies (Noguchi-Shinohara *et al.*, 2009; Treglia and Cason, 2012; Yoshita *et al.*, 2015; Tiraboschi *et al.*, 2016). Although this is in keeping with the male preponderance of DLB observed in in the same geographical region, the proportion is considerably greater than the 55.6% cited in our epidemiological study (Chapter 5).

In summary, a range of factors may have contributed to the difference between the optimum HMR cut-off in our study and those used in other MIBG studies. We used different collimators to those used many other MIBG studies, but our *post hoc* cut-off was lower than the *a priori* cut-off, rather than higher as would be expected if attributable to collimator choice. This would suggest that differences between our general population and those in other countries, such as obesity and ischaemic heart disease, may account for differences between our optimum cut-off and those reported elsewhere.

9.6 Relationship between early and late HMR

As previously discussed, we employed late HMR as the primary outcome measure for cardiac denervation, a practice in keeping with previous studies. Nevertheless, the potential role of early HMR is an important topic given our focus on the clinical utility of MIBG.

Early images in this study were taken 20 minutes after radioisotope injection and late images were taken 180 to 240 minutes after injection. FP-CIT images were gathered between 240 and 360 minutes after each participant's arrival. Any investigation able to provide comparable accuracy to FP-CIT in a shorter period of time would be preferable to both patient and service provider, potentially allowing a higher turnover of patients, while also minimising distress to a vulnerable population.

Usage of early HMR, using the same cut-off, would have only marginally decreased MIBG sensitivity (69%; 95% CI 41- 89%) and increased specificity (77 %; 95% CI 50% - 93%). While this would not have affected overall accuracy of MIBG (73%; 95 CI 55-87%), two cases (one probable DLB, one probable AD) demonstrated abnormal early HMRs but normal late HMRs.

What is notable from the comparison of early and late HMR data is that none of the participants with normal early HMR went on to have abnormal late HMRs when either the *a priori* or *post hoc* cut-off was used. Furthermore, specificity was 100% in patients with abnormal early HMR when the *post hoc* cut-off was used. This suggests that an early HMR <1.7 might be sufficiently suggestive of cardiac denervation in DLB and remove the necessity for collection of late images. While further research could further inform this practice, the potential health economic and patient wellbeing benefits of a considerably shorter hospital visit could make MIBG a more attractive investigation to clinicians.

9.7 MIBG in patients with interacting medications and myocardial infarction

The high proportion (55%) of participants in the study that were prescribed medications identified as capable of interacting with MIBG uptake (Giammarile *et al.*, 2008) is noteworthy in itself. In order for MIBG to become a useful diagnostic tool in clinical practice, it cannot be considered unfeasible in over half of the patient population. Even when dopaminergic medications were excluded, 13 of 33 patients (39%) were prescribed potentially interacting medications. Stopping or holding these medications for the purposes of an investigation would be potentially harmful for patients scheduled for MIBG, particularly given that many of the proposed interactions are theoretical or supported by little or no experimental evidence (Jacobson and Travin, 2015).

The same principles apply to the 15% of participants (5/33) with a history of MI. The UK prevalence of MI is reported as 12.1% in men and 5.5% in women over 75 years old (Bhatnagar *et al.*, 2015). When we consider that cardiovascular risk and MI prevalence is higher in patients with dementia (Norton *et al.*, 2014) and higher in North East England than the UK average (Bhatnagar *et al.*, 2015), excluding patients with ischaemic heart disease from MIBG would exclude an unfeasibly large cross-section of the clinical population for whom the test could be useful.
It is beyond the scope of this study to dismiss the significance of MI or interacting medications, particularly given our small sample size and the small effect sizes observed between groups. Nevertheless, our results are encouraging and support further investigation in this area,

particularly as even a small effect size could have great clinical significance given our observation that HMR cut-offs could greatly influence MIBG utility. A false positive result was suspected in one of our three participants with probable AD and a history of MI, and this case fell only marginally below the 2.2 HMR cut-off *(Table 8.8)*. Were our *post hoc* cut-off to be used, none of these three AD cases would have produced an abnormal MIBG result.

Similarly, an abnormal MIBG result was observed in two of the seven (29%) patients with probable AD that were prescribed an interacting medication; both would have been considered as within normal range were our revised cut-off of 1.7 to be employed *(Table 8.12)*. In both cases, the agent in question was amlodipine, for which approximately 2.4 million prescriptions are made in England every month (Curtis and Goldacre, 2018). However, two other participants with probable AD were prescribed amlodipine and had normal MIBG scans; another patient, prescribed diltiazem, also a calcium channel blocker, demonstrated late HMR within normal parameters.

Four patients with probable DLB (4/17; 24%) were also prescribed calcium channel blockers, two of whom underwent false negative MIBG scans; one was prescribed amlodipine, one prescribed diltiazem. Thus, of nine patients participating in the study with either false positive or false negative MIBG, four (4/9; 44%) were prescribed a calcium blocker, compared to five of 24 participants with true positive or true negative results (5/24;21%). Despite the theoretical expectation that calcium channel blockade would suppress sympathetic activity, clinical studies of amlodipine have reported an increase (Inui *et al.*, 2014) or no significant effect on HMR, albeit in patients with cardiac disease demonstrating an increase in sympathetic activity in response to treatment (Sakata *et al.*, 1998).

A multitude of factors related to calcium channel blocker therapy, and drug prescription in general, could influence HMR. These include, but are not limited to, drug dose, frequency, compliance, and interpatient pharmacokinetic variability. Our data does however, at the very least, cast doubt on any suggestion that such factors should contraindicate, or comprise exclusion criteria from studies of MIBG in DLB populations. Nevertheless, any clinical conclusions from MIBG results in patients prescribed calcium channel blockers should continue to be drawn with caution.

We found that the *post hoc* cut-off of 1.6 providing the optimum diagnostic accuracy in patients prescribed medications thought to interfere with cardiac MIBG uptake was marginally lower than the 1.7 optimum cut-off identified for cohort as a whole. The small sample size of this subgroup should lead this finding to be interpreted with caution, particularly as a single AD case (MID039, *Table 8.11*) would be interpreted as false positive were the 1.7 cut-off to be used. However, the finding might suggest that an adjusted HMR cut-off may be a possible alternative to both compromising clinical utility and withdrawing potentially interfering medications.

9.8 MIBG in patients without parkinsonian symptoms

As previously discussed (*Chapter 3.3.4*), clinical and neuropathological studies have identified the presence of patients with DLB who fail to demonstrate parkinsonian symptoms throughout the course of their illness, and those who, despite the presence of neocortical Lewy body pathology, show little or no striatal pathology *post-mortem* (Harvey, Skelton-Robinson and Rossor, 2003; Ferman *et al.*, 2006; Savica *et al.*, 2013; Zaccai *et al.*, 2015; Walker *et al.*, 2016). There is insufficient evidence to clearly demonstrate that FP-CIT has limited sensitivity in cohorts without parkinsonism, but lower levels of dopamine transporter loss in subjects without extrapyramidal symptoms (Piggott *et al.*, 1999) do raise the possibility that alternative biomarkers, such as MIBG, could be useful in such patients.

We therefore further scrutinised MIBG results for a subgroup of patients without parkinsonism, using a UPDRS cut-off score of 15, employed in other studies, to distinguish patients with clinically significant parkinsonism from those without (McKeith *et al.*, 2007). A cut-off of 15 was chosen as the UPDRS motor subscale comprises 14 components, and that a score in each of those components could have been attributable to cognitive or physical comorbidities in normal ageing (McKeith *et al.*, 2007). This is supported by the fact that none of the AD group recruited scored over 10 on the scale.

Four patients (4/17; 24%) with DLB had UPRDS scores under 15, none of whom were treated with dopaminergic medication that could potentially have decreased their score. This could be considered an under-representation of such patients when considered alongside the results from our epidemiological study, which showed that 27% of patients did not have parkinsonism identified at any point of their contact with services (*Chapter 5.3.5*). However, milder parkinsonian

symptoms, less likely to identified in routine clinical practice, would have been identified through the systematic use of UPDRS in our study methodology.

Of these four patients without parkinsonism, two demonstrated HMR values lower than both *a priori* and *post hoc* cut-offs, but in both of these cases had abnormal FP-CIT results. Of the remaining two cases, both had late HMRs over 2.2 and UPDRS under 6; one had an abnormal FP-CIT, the other normal. The second of these cases in particular is important; the sole recruit with a history of probable DLB, diagnosed on the basis of fluctuations and visual hallucinations, but neither an abnormal FP-CIT nor abnormal MIBG. On one hand, this could represent a lack of specificity, as previously discussed, of the third consensus criteria and our process of rating through independent diagnosticians; on the other, it could represent a case of DLB, with cortical α-synuclein pathology but no significant cardiac denervation or striatal involvement. If the latter is to be presumed, and the small sample size taken into consideration, the sensitivity of MIBG in differentiating DLB in patients without parkinsonism from AD is 50% (95% CI 7% - 93%). This clearly does not support the use of MIBG in such patients without further research into this group, particularly as FP-CIT provides higher sensitivity in this group of patients without parkinsonism (75%; 95% CI 19% - 99%).

9.9 MIBG in patients with normal FP-CIT

We recruited six patients meeting criteria for probable or possible DLB, who had recently had a normal FP-CIT scan (*Chapter 8.10*). It was important to consider this group separately from our main cohort, not only because of the different mechanism of recruitment (potential participants were identified after screening of regional neuroimaging databases), but because of the clear differences in demographic and clinical characteristics between the two subsets.

FP-CIT scans are generally requested and conducted in the diagnostic phase of contact with services. This is reflected in patient demographics, where participants recruited from FP-CIT databases trended towards lower scoring in scales measuring cognitive ability, core symptoms, neuropsychiatric symptoms, carer distress and global condition when compared to the main study cohort, despite a higher mean age observed in the former. Participants identified in this diagnostic phase of contact with services could also be less likely to be prescribed medications, such as levodopa, identified as capable of interacting with MIBG.

These participants not only differ from our main study population in demographic and clinical scale measurements, but in having been referred for FP-CIT, they also represent a phenotypically different population, with a higher degree of diagnostic uncertainty, than patients in the main cohort. Clinicians detecting more than one clear core feature need not refer to FP-CIT, as such cases meet criteria for probable DLB with or without an indicative biomarker (McKeith *et al.*, 2005, 2017). This is reflected, if not perhaps underestimated, by the higher proportion of possible DLB cases in our sample identified through FP-CIT screening (2/6; 33%), than in our main cohort (1/17; 6%).

The small sample size in our FP-CIT negative DLB group makes direct comparison with the main cohort difficult, but the significantly lower scores in scales UPDRS in the former is understandable as striatal uptake has been shown to correlate with UPDRS score in DLB and PD populations (Benamer *et al.*, 2000; Del Sole *et al.*, 2015).

What is immediately striking about the results of the group recruited from neuroimaging databases are the FP-CIT results reported; two of six DLB cases (one probable DLB, one possible DLB) underwent FP-CIT scans clinically reported as normal but reported as abnormal by our panel of five practitioners. It should be noted that this reflects two interpretations of the same scan data, rather than two scans conducted separately in the clinical and research settings.

High levels of inter-reader agreement have been observed in FP-CIT raters (Seibyl *et al.*, 2014) and panels of practitioners blinded to clinical data, like the one employed in this study, have demonstrated superior specificity and inferior sensitivity to their non-blinded counterparts (O'Brien *et al.*, 2014b). However, the two normal clinical interpretations, later revised to abnormal, are likely to reflect false negatives; a lower sensitivity by a single non-blinded rater than the methodologically more robust five-person panel adopted by our study.

These two cases, together with the rest of our results, may suggest therefore that MIBG has a role in two types of cases with normal FP-CIT results; those with an insufficient or absent striatal LB pathology; or those reported as normal by a single reader. It may be prudent, therefore, to suggest that FP-CIT negative patients undergo a secondary review of study images, or semi-quantitative analysis, prior to recommendation or authorisation of subsequent MIBG study. This is particularly pertinent when one considers that the majority of FP-CIT research has been conducted

near DLB research centres, using 3-5-person rater panels; the accuracy of the modality is likely to decrease among single raters in other settings.

Abnormal MIBG results were reported in two of the six participants (33%) identified through our FP-CIT screening process, both in cases meeting criteria for probable DLB. The first such participant had both a history of MI and of prescription of interacting medication (diltiazem), who despite falling under our *a priori* threshold would be reported as a normal scan were our *post hoc* to be employed. Furthermore, this participant comprised one of two cases described above; FP-CIT initially reported as normal but revised to abnormal upon blinded panel rating. This therefore represents a case where both FP-CIT and MIBG individually represent a high degree of uncertainty, but when combined lend considerable support to DLB diagnosis.

The second of the cases with abnormal MIBG, recruited using neuroimaging databases, had neither a history of MI nor of interacting medications, and had a negative FP-CIT; late HMR for this patient was lower than both *a priori* and *post hoc* cut-offs. A case sharing each of these characteristics was also recruited to our main study using our conventional clinical screening mechanism. We therefore identified two DLB cases with abnormal MIBG results, suggesting the presence of marked cardiac sympathetic pathology (late HMRs of 1.4 and 1.5) in the absence of striatal pathology sufficient to produce an abnormal FP-CIT.

9.10 MIBG and DLB neuropathology

The combinations of clinical, FP-CIT and MIBG findings among patients participating in our main cohort (*Figure 9.1*) appear to contradict the Braak hypothesis of LB pathogenesis (Braak *et al.*, 2003). According to this hypothesis, the spread of α-synuclein disease through the nervous system should be sequential and predictable. Peripheral nervous system pathology, measured in this study using MIBG as a marker of cardiac sympathetic denervation, should precede striatal involvement, measured using FP-CIT, which should in turn precede cortical disease. However, three DLB patients recruited to the main cohort exhibited findings contrary to the ascending pattern proposed by the Braak hypothesis, demonstrating normal MIBG scans, indicative of an absence of cardiac sympathetic pathology, but abnormal FP-CIT findings, suggesting the presence of striatal LB pathology (*Table 9.1*).

This data joins a growing body of evidence disputing the Braak theory of pathogenesis. Yokota *et al*. (2007) identified advanced limbic and cortical LB pathology in two cases without parkinsonism, both with a degree of epicardial involvement and intact but relative preservation of the brainstem. Zaccai *et al*. (2015) reported that only 39.3% of a sample of 208 community-recruited participants demonstrated patterns of pathology concurrent with the Braak hypothesis, with 21.4% adhering to an amygdala-predominant pattern and 39.3% demonstrating an atypical distribution of disease.

These neuropathological findings are supported by the large number of observational studies reporting that less than 85% of patients with DLB demonstrate parkinsonian symptoms during the course of their illness (Harvey, Skelton-Robinson and Rossor, 2003; Ferman *et al.*, 2006; Savica *et al.*, 2013; Walker *et al.*, 2016). These patients are therefore less likely to have striatal α-synuclein pathology, or at least sufficient pathology to be detected by FP-CIT. Although a proportion of such subjects may be detectable if more sensitive measures of striatal function were available, Thomas *et al*. (2017a) recently reported that 10% of DLB cases exhibited substantial LB disease in cortical and limbic areas without substantial levels of striatal disease.

Despite several studies investigating the use of MIBG in differentiating DLB from non-DLB dementia, relatively few neuropathological studies have explored the prevalence of cardiac sympathetic involvement in LB disease. Orimo *et al*. (2005) observed loss of tyroxine hydroxylase immunoreactive nerve fibres, a marker for sympathetic denervation, in all 11 patients with DLB or mixed DLB/AD disease. This was followed by work from the same group that identified α-synuclein aggregates in 18 of 20 patients with incidental Lewy body disease and 6 of 10 with PD (Orimo *et al.*, 2008), a finding proposed to suggest distal sympathetic degeneration preceded clinical PD and progression to more proximal structures.

More recently, Takahashi *et al*. (2015) published the results of the only study to date comparing MIBG uptake directly with neuropathological data, finding a significant correlation between HMR and degree of cardiac axonal degeneration. Notably, the study did identify two of 13 cases meeting neuropathological criteria for DLB that had no decrease in MIBG uptake but did demonstrate at least a degree of sympathetic denervation. This is important to consider in the context of our MIBG false negative DLB cases; despite normal HMR values they may have cardiac α-synuclein pathology. This shows consistency with the finding that DLB subjects with cortical and striatal α-synuclein pathology, but without significant nigrostriatal loss, may not demonstrate

sufficient deficits in FP-CIT uptake to produce an abnormal scan result (Colloby *et al.*, 2012). This may suggest that a marker that measured α-synuclein directly, rather than dysfunction associated with α-synuclein, could provide a higher diagnostic accuracy than that provided by both FP-CIT and MIBG.

Generalising the scarce neuropathological data to our findings is challenging. All of the aforementioned studies have taken place among Japanese populations and data among other groups have produced more modest rates of aggregation in cardiac tissues; Beach *et al*. (2010) detected cardiac α-synuclein pathology in only one of four DLB cases undergoing multi-organ *post-mortem* examination. As previously discussed, all neuropathological studies can be subject to recruitment bias (Fillenbaum *et al.*, 1996; Tsuang *et al.*, 2006; Zaccai, Ince and Brayne, 2006) and consideration of must also be drawn to the younger mean age of these study cohorts. The superior sensitivity (95.7%) of MIBG in Takahashi *et al*'s study to that reported in the majority of clinical studies could also suggest that this sample was less clinically representative than that seen in our study (Takahashi *et al.*, 2015).

Our data, in demonstrating evidence of cardiac denervation in a range of patients with different combinations of clinical phenotypes and FP-CIT imaging statuses, represent *in vivo* imaging findings that support the body of evidence disputing the Braak hypothesis. However, more research is needed to understand the relationship in particular between MIBG myocardial uptake and histopathological lesions before the prevalence and temporality of cardiac denervation in DLB can be further understood.

9.11 Strengths and weaknesses

We report clinically relevant and generalisable findings from what we believe to be the first study examining MIBG utility in a UK cohort. In including patients with a history of MI, and those prescribed medications identified as capable of interfering with MIBG uptake, ours constitutes a more clinically representative sample than those seen in previous studies. In keeping with our aims, the study identified a feasible cut-off point for future studies, demonstrating excellent specificity and satisfactory sensitivity.

The prevalence of both MI and potentially interacting medications underline the importance of understanding the relationship between these factors and MIBG uptake, and in particular our data has added to a relatively sparse group of human studies examining the significance of calcium channel blockers in late HMR.

The process by which clinical diagnoses were assigned was robust and replicable, as was the methodology for reporting of the FP-CIT data. Our findings relating to abnormal early HMR data raises important economic questions for services and if supported by further evidence could comprise an important factor for clinicians considering use of MIBG.

Varying combinations of clinical diagnosis, FP-CIT and MIBG and our findings in an FP-CIT negative cohort provide a clinical insight into DLB pathogenesis and support a growing body of evidence disputing the Braak hypothesis.

Our study was not without limitations. The small sample size limited the power of analyses and while providing a degree of reassurance to clinicians regarding these factors it fell considerably short being able of offer firm conclusions. This was the case in particular regarding MI and interfering medications and the small effect sizes observed groups in these analyses. Our sample size was a product of the challenges of identifying and recruiting DLB patients and the time constraints to which the study was bound. These also likely contributed to a selection bias; every patient with DLB diagnosed within the region within the study recruitment phase was likely to have been considered as a candidate for participation. This was not the case for AD patients, who having been drawn from a much larger pool, were more likely to have volunteered for participation through local registers or referred from other studies.

9.12 Summary

Our findings support the potential of MIBG as a diagnostic biomarker in differentiating DLB from AD in a clinical population. An optimum sensitivity for DLB of 71% and specificity for AD of 100% suggest moderate clinical utility but underline the importance of an appropriate cut-off, necessitating calibration and consideration of technical and patient-based factors. Our failure to identify significant relationships between HMR and both MI and interacting medications is encouraging regarding the feasibility of MIBG, but unquestionably requires further research before dismissing the role of these factors. In particular, the role of calcium channel blockers,

prescribed frequently in older adults and capable of producing false negative and false positive MIBG results, merits further exploration.

Our observation that DLB patients can exhibit normal MIBG results and abnormal FP-CIT findings would appear to add to a body of evidence challenging the Braak hypothesis of LB disease propagation. However, a greater understanding of the relationship between HMR and LB pathology must be achieved before MIBG findings can be extrapolated to contribute to models of pathogenesis with greater certainty.

Chapter 10

Conclusions and directions for future research

This thesis has explored the detection of DLB in routine clinical care and the impact that the diagnosis has on patients' contact with secondary care services. It investigated the role of the diagnostic biomarkers FP-CIT and cardiac MIBG in enhancing clinical DLB diagnosis. Future research should consider both improving detection of clinical features and the use of biomarkers in optimising DLB case recognition in routine secondary care.

10.1 Summary of main findings

The first half of this thesis concerned the clinical epidemiology of DLB. It also explored the contact that DLB cases and matched dementia controls had with secondary care services. Our cross-sectional survey of 5 569 patients seen in POA services found that DLB represented 5.6% of dementia cases and was more common among males and younger age groups. We observed a range of DLB prevalence rates between neighbouring services, which may be more closely related to differences in disease detection rather than in the true prevalence of pathology; it seems unlikely that regions and services so geographically close could be subject to pronounced variation in environmental factors. This range in detection may explain the variation in DLB prevalence rates seen in previous studies (Vann Jones and O'Brien, 2014).

Over a third of DLB cases were assigned a different dementia subtype diagnosis during their initial contact with secondary care, and patients with DLB encountered a significantly longer time from presentation to final diagnosis (median 265 days) than that seen in other dementia subtypes (median 125 days). During the course of their care, patients with DLB also had more extensive contact with clinical services (median 17 contacts) than those with non-DLB subtypes (10 contacts) and had a higher frequency of appointments with specialist nurses (median 11 appointments (DLB) *vs* 3 appointments (non-DLB)) and medical practitioners (median 7 appointments (DLB) *vs* 5 appointments (non-DLB)).

We observed that clinicians routinely used biomarkers throughout the diagnostic process; nearly half of all DLB patients were referred for FP-CIT scans. Of core and suggestive DLB symptoms, only visual hallucinations were recorded more frequently than impaired striatal uptake on FP-CIT

scanning, suggesting that FP-CIT plays an important role in the diagnostic process. Patients with DLB who had FP-CIT scans experienced a significantly longer time from referral to initial dementia diagnosis (median 211 days) than those that did not (100 days). This may suggest that waiting times for FP-CIT, and the wait for a subsequent POA appointment at which to discuss the results of the scan, may be a factor in delaying initial DLB diagnosis. There was also a non-significant trend for patients in this FP-CIT group to experience a shorter period of time from referral to final diagnosis (median 245 days) when compared to the non-FP-CIT group (358 days). This may suggest that the waiting time associated with FP-CIT may be offset in part by a longer time for revision of diagnosis in patients who did not undergo FP-CIT, and quicker access to biomarkers such as FP-CIT may shorten the time to final diagnosis in DLB patients.

The second half of the thesis therefore explored how DLB case detection might be enhanced through the introduction of a second DLB biomarker; MIBG cardiac scintigraphy. We investigated the utility of MIBG for differentiating DLB from AD in a clinically representative population. Using a HMR cut-off derived from the results of a Japanese multicenter study (Yoshita *et al.*, 2015), we found that MIBG had a moderate sensitivity (71%) and specificity (75%) for differentiating DLB from AD. However, we found that when an optimum threshold, as determined by ROC analysis, was used to differentiate abnormal scans from normal ones, the specificity for AD of MIBG was enhanced (100%), without compromising sensitivity for DLB (71%). MIBG diagnostic accuracy is therefore highly dependent on the choice of cut-off used, and factors affecting different populations may influence this cut-off. We found that a high proportion of our participants possessed clinical characteristics that would have excluded them from previous studies on the basis that these might adversely affect clinical utility; a history of MI (15%), and the prescription of medications thought to interfere with MIBG uptake (55%). We identified no significant relationships between these characteristics and HMR, which encourage further research into MIBG using clinically representative populations.

10.2 Directions for future research

Although we believe variation in DLB prevalence rates between different services to be related to detection of disease rather than true variation in the pathological changes associated with DLB (Kane *et al.*, 2018), there remains an important role for further research into the clinical epidemiology of the condition. This includes research that uses designs that identify DLB on the

basis of clinical symptoms, even with the expectation that such cohorts will fail to include a proportion of cases with neuropathological DLB.

Further research is also needed into DLB prevalence rates in different clinical services. We included a range of services that we believed to be representative of those that comprise NHS POA services throughout the UK. Although only one of the services included had close links with Newcastle University, proximity alone to a research centre may produce a greater awareness of DLB that may result in higher rates of diagnosis (Bonanni *et al.*, 2013). Epidemiological studies that include a more heterogenous group of services and regions may further aid understanding of the clinical factors underpinning DLB diagnosis.

Such research could be facilitated by the development of electronic care records, which enable retrospective access to large, clinically representative populations of anonymised cases suitable for epidemiological research (Fernandes *et al.*, 2013). Such studies are vulnerable to the effects of incomplete or unclear data (Goodman *et al.*, 2017), but are feasible, and would allow observation of changes in prevalence over time, assuming consistency of diagnostic practice.

The support lent by our data to the hypothesis that detection, rather than true disease prevalence, is responsible for differences in case frequency emphasises the need for research studies into enhancing clinical DLB case detection, such those investigating diagnostic scales and toolkits (Galvin, 2015; Thomas *et al.*, 2017). Systematic use of such toolkits in non-DLB populations could identify their potential to detect DLB cases otherwise missed in routine clinical care.

Our finding that patients with DLB received more extensive contact with secondary care than those with non-DLB dementias has important implications for resource allocation and should be followed by studies exploring the economic impact of DLB compared with that of other dementia subtypes, and in particular, whether processes allowing earlier or more accurate diagnosis, would be more cost-effective. This is important when one considers the cost of biomarkers in DLB diagnosis, and our finding that time from referral to initial diagnosis may have been prolonged in DLB patients by waiting for FP-CIT scans. Diagnostic revision may also come with an opportunity cost to clinical issues like pharmacological management; early and accurate DLB diagnosis could

allow for a more assertive, proactive approach to treatment, and more studies on the clinical outcomes of DLB detection should be pursued.

Our data regarding MIBG utility are encouraging but suggest that further work is required before the biomarker can feasibly be integrated into routine clinical practice. MIBG studies to date have been limited by either non-representative populations or, as in our case, smaller sample sizes. An important next step in determining MIBG utility is therefore a multicentre study in a clinically representative population, particularly as larger sample sizes will be important in identifying and validating suitable HMR cut-offs, and in adequately clarifying the issues discussed below.

Our study did not identify overall group differences in HMR between patients administered and not administered potentially interacting medications, but a future, larger multicentre study could allow subgroup analysis of different drug classes to further investigate this. Other study designs, such as case series reporting serial MIBG on individual patients, could also be helpful in clarifying the effects of such medications and are not unprecedented (Sisson *et al.*, 1987; Apeldoorn, Voerman and Hoefnagel, 1995). A multicentre study would also allow subgroup analysis of patients with comorbidities, but other study designs may also be helpful in this regard. Using SPECT-CT to understand how regional decreases in cardiac MIBG relate to overall HMR (Odagiri *et al.*, 2016), and correlation of HMR with cardiac neuropathological findings (Orimo *et al.*, 2016) would further support use of the biomarker in patients with ischaemic heart disease. Comparison of MIBG data with neuropathological findings, the gold standard for DLB diagnosis (McKeith *et al.*, 2017), will be a crucial step in validating MIBG as a biomarker.

Even if validated by neuropathological findings, other considerations must be made before MIBG can be considered alongside FP-CIT as a first line investigation for DLB. This is reflected in revised UK clinical guidelines for the investigation of dementia, which recommend MIBG only when FP-CIT is unavailable (NICE, 2018). Among important areas for future research are technical considerations, such as the identification of appropriate HMR thresholds, which as demonstrated by our study, have important implications for MIBG utility. This would best be achieved by the development of calibration protocols for MIBG (Nakajima *et al.*, 2012; Verschure *et al.*, 2017) and the creation of international MIBG databases (Nakajima *et al.*, 2016). It is worth noting that FP-CIT images are acquired using SPECT methods, and considerable research has been devoted to optimisation of the processing and quantifying of these images. The current methods of

acquisition, processing and analysis of MIBG images is relatively crude by comparison, and further technical research in this area is warranted. A greater knowledge of how normal ageing affects MIBG uptake, and how much variation in HMR could be expected in both healthy populations and dementia cohorts, will be important in determination of suitable HMR cut-offs for use in routine clinical practice. Further work should also be conducted on interrater agreement in MIBG (Veltman *et al.*, 2012; Yoshita *et al.*, 2015).

Eventual integration of MIBG into clinical practice should be done so with consideration of optimising the combined utility of FP-CIT and MIBG. Larger sample sizes may help in identifying phenotypes associated with higher diagnostic utility, and therefore direct clinicians towards the appropriate investigation in patients with particular clinical symptoms. Such algorithmic approaches have been previously proposed for the use of FDG PET in DLB investigation (Firbank *et al.*, 2016). One such group that may benefit from this approach is patients meeting criteria for prodromal DLB (McKeith *et al.*, 2016).

Further research is also required into the pathogenesis and temporal development of pathology in DLB. We identified three DLB participants with insufficient cardiac α-synuclein pathology to produce an abnormal MIBG result, but with striatal degeneration capable of producing abnormal FP-CIT findings. This pattern challenges the Braak hypothesis of DLB pathogenesis (Braak *et al.*, 2003) that has also been contradicted by community neuropathological samples (Zaccai *et al.*, 2015).

A greater understanding of DLB pathogenesis might be supported by further research into how and when α-synuclein pathology affects the peripheral nervous system in DLB as this may lead to other diagnostic markers. The colon, submandibular gland, and skin have all been investigated as practicable biopsy sites capable of demonstrating evidence of α-synuclein pathology, but studies have mostly been confined to single-centre designs (Lee *et al.*, 2017). The suggestion made by the Braak hypothesis that such peripheral pathology occurs earlier than that in the CNS (Braak *et al.*, 2003) encourages work into the use of biopsy markers in patients with prodromal DLB. Such biopsy data would be most valuable when collected at multiple time points, and compared with other biomarkers, such as MIBG and FP-CIT, and clinical signs and symptoms. This could also be supported by more widespread investigation of peripheral neuropathology, and comparison with

CNS pathology in *post mortem* samples.

Chapter 11

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